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AN EXPERIMENTAL STUDY OF TESTS FOR THE DETECTION OF CARRIERS OF BACTERIUM PULLORUM

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INTRODUCTION

A review of the literature of the last decade shows that investigators are far from being in unanimous agreement regarding the value of tests for the detection of fowls that are carriers of *Bacterium pullorum*. Following the findings of Jones(1) in 1912, that the agglutination test was of value for this purpose, this test came into extensive use in some sections of the country.

The rather complicated procedure of performing the agglutination test has undoubtedly been an important factor in retarding its more general employment throughout the United States for the eradication of *Bacterium pullorum* infection in breeding fowls. In addition, the accuracy of the test has recently been questioned by several investigators, particularly B. A. Beach, Halpin, and Lampman,(2) J. R. Beach,(3) Fitch and Lubbehusen,(4) and Newsom, Cross, and Ufford,(5) who have shown conclusively that a negative reaction to the agglutination test does not always indicate freedom from *Bacterium pullorum* infection. These factors have stimulated attempts to develop a test of equal or greater accuracy but simpler in application than the agglutination test.

In 1917, Ward and Gallagher(6) reported upon an intradermal test for detecting the carriers of *Bacterium pullorum*. These investigators used a broth culture of *Bacterium pullorum*, which was incu-

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bated for about a month, carbolized, and stored for several weeks longer. This was injected intradermally into the margin of the wattle. A positive reaction consisted of a swelling of the injected wattle which persisted for at least twenty-four hours. Similar experiments have since been conducted by a number of investigators, including Scherago and Benson,[7] J. R. Beach,[8] Fuller,[9] Brunett,[10] Cernaianu,[11] Edington,[12] Broerman,[13] Graham and Tunnicliff,[14, 15] Edwards and Hull,[16] Gwatkin,[17] Stafseth and Thorp,[18] Bushnell and his associates,[19, 20, 21] B. A. Beach,[22] and Owen.[23] Interest in the intradermal test was further stimulated in 1926 and 1927, when various manufacturers of biologic preparations began marketing, under the name of 'pullorin' products for testing poultry for *Bacterium pullorum* infection by the intradermal method. Variable results have been reported by the different investigators, but their findings for the most part indicated that the intradermal test was less satisfactory than the agglutination test.

With few exceptions, the experiments previously mentioned were concerned with one type of preparation for intradermal use and, consequently, studies of the relative value of different types of such preparations have been lacking. Furthermore, the result of the agglutination test has been the principal criterion of the merits of the intradermal test, whereas this should more properly consist of the bacteriological findings alone. It was with these considerations in mind that the studies reported herein were undertaken.

METHODS USED IN THE EXPERIMENTS AT THE CALIFORNIA AGRICULTURAL EXPERIMENT STATION

In these experiments four types of preparation for intradermal testing were used, i.e., concentrated, precipitated, cell suspension, and cell solution. For the sake of brevity, these will be designated by the proprietary name of 'pullorin'. Part of the pullorins employed were secured from outside sources and part were prepared in our laboratory. Some of the fowls that were tested came from commercial flocks; others were of those used at the Experiment Station. All the birds were subjected to the intradermal and agglutination tests simultaneously and were later destroyed for post-mortem and bacteriological examinations.

The pullorin was injected intradermally into the lower margin of the right wattle. An attempt was made to produce a swelling, about the size of a wheat kernel, at the point of injection. The readings

were made after twenty-four hours. The scheme of reading intradermal reactions followed by Graham[3] in his experiments with the pullorin test was used. This is illustrated in figure 1.

The agglutination tests were set in dilutions of 1–25, 1–50, 1–100, and 1–200. Complete agglutination in any one of the four tubes was considered as a positive reaction.[4]

Upon completion of the intradermal and agglutination tests, the fowls were killed and examined. Lesions occurring in any organ were noted and cultured and, in addition, cultures were made of heart

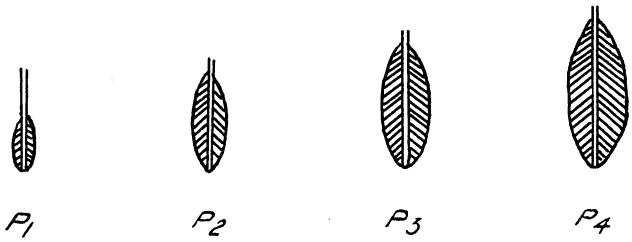

Fig. 1. Illinois Experiment Station diagram of reactions to pullorin. The clear center space indicates the thickness of a normal wattle. The shaded portion indicates the swelling resulting from the injection of pullorin.

blood, liver, and ovaries (of hens) or testicles (of males), whether or not lesions were present. Each culture was made in duplicate in bouillon and cooked blood agar. All the cultures which presented Gram-negative rods were planted in glucose, mannite, maltose, saccharose, and lactose broth, each containing 1 per cent Andrade's indicator. Those that fermented glucose and mannite only were recorded as *Bacterium pullorum;* strains producing acid alone were termed anaerogenic, those producing both acid and gas, aerogenic.

Only pullorin of the precipitated or powdered type was secured from an outside source. This was dissolved in sterile water or sterile water containing 0.5 per cent phenol, just before use. Part of the pullorin was purchased from a commercial laboratory and part was furnished by Dr. Robert Graham of the Illinois Agricultural Experiment Station.

[3] Graham, R., Personal communication, 1928.

[4] *Technique of the agglutination test.*—A culture (No. 9) of *Bacterium pullorum* of known good agglutinating qualities was used for the antigen for all of the tests. This antigen was prepared by washing off a 48-hour growth of *Bacterium pullorum* in agar flasks with 0.85 per cent saline solution containing 0.5 per cent phenol; 1 cc of N_1 NaOH was added to 100 cc of antigen, according to Mallman's[24] recommendation, to eliminate cloudy reactions. It was standardized to the density of 4.5 cm by the Gates' nephelometer.

The tubes were incubated at 37° C for 24 hours. First readings were made after this period. They were then held at room temperature for another twenty-four hours and, at the termination of this time, the final reading was made.

TESTS WITH THE COMMERCIAL PULLORIN

Eighty-five white leghorn hens, secured from a poultryman, were tested with this product and by the agglutination test, and were killed and examined after the tests were completed. All of the hens had previously reacted to an agglutination test. The results are given in table 1.

TABLE 1

COMPARISON OF THE RESULTS OF TESTS WITH A COMMERCIAL PRECIPITATED PULLORIN AND BY THE AGGLUTINATION METHOD, AND OF POST-MORTEM EXAMINATION OF 85 HENS

Results of pullorin tests	Results of agglutination tests	Macroscopic lesions present	Bacterium pullorum isolated	Number of fowls	Per cent of total fowls
+[1]	+	+	+	52	61.1
+[2]	+	+[3]	−	2	2.3
+[4]	+	−	−	1	1.1
+[5]	−	+	−[6]	3	3.5
+[7]	−	−	−	5	5.8
−	+	+	+	18	21.1
−	−	−	−	4	4.7

[1] The reactions of 35 fowls were recorded as P_1, of 13 as P_2, and of 4 as P_3. The swelling involved both wattles of one of the P_3 fowls.

[2] The reaction of 1 fowl was recorded as P_1, of 1 as P_2.

[3] Abnormal yolks found in one, fibrinous pericarditis in the other.

[4] The reaction of this fowl was recorded as P_1.

[5] The reactions of 2 fowls were recorded as P_1, of 1 as P_2.

[6] *Bacillus coli* obtained in cultures from abnormal yolks of one bird and from a small abdominal cyst of another. The third bird had an abdominal cyst, the culture from which remained sterile.

[7] The reactions of all five fowls were recorded as P_1.

The data presented in table 1 show a marked discrepancy between the results of the pullorin tests and the bacteriological findings. Such discrepancy, but much less marked, is also seen with respect to the results of the agglutination test. This is brought out more clearly by further summarizing of the data. Thus, of the 70 bacteriologically positive fowls, 52, or 74.2 per cent, were detected by the pullorin test, while all of them gave a positive reaction to the agglutination test. Of the 15 bacteriologically negative fowls, 11, or 73.3 per cent, reacted to the pullorin test, and 3, or 20 per cent, reacted to the agglutination test. These results indicate that the reactions that resulted from the intradermal injection of this pullorin were, to a large extent, nonspecific. Another objectionable feature of the pullorin tests is that with 43 of the 63 positive reactions, the degree of reactions was slight (P_1). This made a definite reading difficult in many cases.

TESTS WITH THE PULLORINS OBTAINED FROM THE ILLINOIS AGRICULTURAL EXPERIMENT STATION

Five lots of powdered precipitated pullorin were received from this source. These were designated as Nos. 27, 28, 30-A, 5400-E, and 5463-F. Two hundred and thirty-five fowls were tested with these pullorins and by the agglutination method, and were later killed for post-mortem examinations.

The fowls consisted partly of hens secured from poultry farms and partly of 3 to 4-months-old White Leghorn cockerels. Some of the hens were reactors from flocks which had been previously tested by the agglutination method and some were birds that were culled from flocks because of non-productiveness. The cockerels were expected to be free from *Bacterium pullorum* infection, but were included when all of a group of hens to be tested had previously reacted to the agglutination test in order to provide adequate controls for the specificity of the reactions resulting from the injection of pullorin. The results are given in table 2.

In table 2, it is seen that there was very close agreement between the results of the tests with pullorins 27 and 28, the results of the agglutination tests, and the results of the post-mortem and bacteriological examinations of the 92 fowls. The results from the other three pullorins, however, were less satisfactory.

Pullorin 30-A detected 18, or 75 per cent, of the 24 bacteriologically positive fowls and caused a reaction in 8, or 21.6 per cent, of the bacteriologically negative fowls. The results of the agglutination test and the bacteriological examination of the same fowls were in perfect agreement.

Pullorin 5400-E caused a reaction in 12, or 92.3 per cent, of the 13 bacteriologically positive fowls and also in 20, or 58.8 per cent, of the 34 bacteriologically negative fowls. One of the bacteriologically negative fowls reacted to the agglutination test, but otherwise the results of the agglutination tests and bacteriological examinations of the 61 birds coincided.

Pullorin 5463-F caused a reaction in all of the 11 bacteriologically positive fowls and also in 15, or 62.5 per cent, of the 24 bacteriologically negative fowls. With the exception of the reaction of one bacteriologically negative fowl, there was perfect agreement between the results of the agglutination tests and the bacteriological findings.

From the preceding, it is seen that there was a marked variation in the performance of the five lots of the same type of pullorin from the

same source. The reactions produced by two of them appeared to be highly specific in detecting carriers of *Bacterium pullorum*. The other three, however, exhibited such a high degree of non-specificity of reaction that they could not be regarded as of much value for that purpose.

TABLE 2

RESULTS OF TESTS WITH ILLINOIS AGRICULTURAL EXPERIMENT STATION PULLORIN, AND BY THE AGGLUTINATION METHOD, AND OF POST-MORTEM EXAMINATION OF 235 FOWLS

Pullorin No.	Number of fowls tested	Results of pullorin tests	Results of agglutination tests	Macroscopic lesions present	Bacterium pullorum isolated	Number of fowls	Per cent of fowls
27	43	+[1]	+	+	+	24[2]	55.8
		−	+	+	−[3]	1[2]	2.3
		−	−	−	−	18[4]	41.8
28	49	+[5]	+	+	+	22[2]	44.9
		+[6]	+	+[7]	−[7]	1	2.0
		+[8]	−	+	+	1	2.0
		−	+	+	+	2	4.1
		−	−	−	−	23[4]	46.9
30-A	61	+[9]	+	+	+	18[2]	29.5
		+[10]	−	−	−	8[11]	13.1
		−	+	+	+	6[2, 12]	9.8
		−	−	−	−	29[13]	47.5
5400-E	47[16]	+[14]	+	+	+	12	25.5
		+[15]	+	−	−	1	2.1
		+[17]	−	−	−	19	40.4
		−	+	+	+	1	2.1
		−	−	−	−	14	29.7
5463-F	35[16]	+[18]	+	+	+	11	31.4
		+[19]	+	+	−	1	2.8
		+[20]	−	−	−	14	40.0
		−	−	−	−	9	25.7

[1] 8 reactions recorded, as P_1, 13 as P_2, 3 as P_3.
[2] Fowls that had reacted to a previous agglutination test.
[3] *Bacillus coli* isolated from abnormal yolks.
[4] Control cockerels.
[5] 10 reactions recorded as P_1, 10 as P_2, 2 as P_3.
[6] Reaction recorded as P_1.
[7] Lesions consisted of cysts attached to the oviduct. *B. coli* isolated from cysts.
[8] Reaction recorded as P_2.
[9] 7 reactions recorded as P_1, 7 as P_2, 4 as P_3.
[10] 4 reactions recorded as P_1, 4 as P_2.
[11] Includes 4 hens and 4 control cockerels.
[12] Included 1 old male. Testes small and hard. *Bacterium pullorum* isolated from testes.
[13] 3 hens and 26 control cockerels.
[14] 4 reactions recorded as P_1, 3 as P_2, 4 as P_3, and 1 as P_4.
[15] Reaction recorded as P_1.
[16] All hens that had been culled because of non-productiveness. Not previously tested.
[17] 9 reactions recorded as P_1, 8 as P_2, 2 as P_3.
[18] 3 reactions recorded as P_1, 4 as P_2, 3 as P_3, 1 as P_4.
[19] Reaction recorded as P_2.
[20] 10 reactions recorded as P_1, 4 as P_2.

The degree of reactions produced by these pullorins was, in a majority of instances, sufficient so that the readings could be easily made, 56 of the 88 positive reactions of the bacteriologically positive birds and 19 of the 44 reactions of the bacteriologically negative birds being recorded as P_2 or greater. In this respect, the performance of these pullorins was more satisfactory than that of the commercial pullorin.

TESTS WITH PULLORINS PREPARED AT THE CALIFORNIA AGRICULTURAL EXPERIMENT STATION

For these tests four types of pullorin were prepared. These are concentrated, precipitated, cell-suspension, and cell-solution, and are designated as pullorin *A, B, C,* and *D,* respectively. The methods of preparation are as follows:

Pullorin A (Concentrated).—This was prepared by the method of Graham,[5] which is similar to the method of preparation of tuberculin. *Bacterium pullorum* was grown in bouillon for a period of two months. At the end of this time, it was tested for purity, killed by heating for one hour at 60° C, filtered through sterile cotton, and the filtrate reduced to one-tenth of its original volume by heating over the water bath at 80° C, and autoclaved for 20 minutes at 15 pounds pressure. To the concentrated pullorin, an equal amount of 0.85 per cent sterile salt solution was added just before making the intradermal test.

Pullorin B (Alcohol Precipitate).—Concentrated pullorin for this purpose was kindly furnished by Dr. F. W. Wood of the Cutter Laboratories, Berkeley. It had been prepared by growing *Bacterium pullorum* in glycerin-bouillon for a period of 3 months. Precipitation was carried out by the method suggested by Graham[6] in making powdered pullorin. One part of concentrated pullorin was added to 20 parts of absolute alcohol, the precipitate washed three times in absolute alcohol and three times in sulphuric ether, and dried in jars over calcium chloride. For intradermal tests, 20 per cent solution of the powdered pullorin by weight in sterile 0.85 per cent salt solution containing 0.5 per cent phenol was used. The solution was slightly cloudy and amber in color.

Pullorin C (Cell-Suspension).—This product was obtained by washing 48-hour agar cultures of *Bacterium pullorum* in sterile 0.85 per cent salt solution. The organism used was an aerogenic strain (No. 9) originally isolated from a baby chick. After washing and

[5] Graham, R. Personal communication, 1928.
[6] Graham, R., Personal communication, 1928.

centrifuging three times the sediment was re-suspended in sterile 0.85 per cent salt solution, containing 0.5 per cent phenol. The heavy suspension was diluted and standardized by means of the Gates' nephelometer. Four dilutions were made with respective densities of 1, 2, 3, and 4 cm. The four bacterial suspensions were placed in the Arnold sterilizer at 60° C. At the end of one hour, they were removed, cooled, and tested for sterility by inoculating a few drops of the suspension into tubes of bouillon and blood agar. These were then incubated at 37° C for 24 hours and were found to be sterile.

Pullorin D (Cell-Solution Product).—The technique in the preparation of this pullorin is the modification by Schoenholz and Meyer[25] of the procedure suggested by Zinsser in the preparation of tuberculin and abortin. In the preparation of pullorin, certain deviations were made from the technique of the first two workers. The 72-hour growth of *Bacterium pullorum* on blake bottles containing agar was washed off with 0.85 per cent salt solution, centrifuged and washed three times, and re-suspended in sterile, distilled water. At this time it was tested for purity. The density of the suspension was measured by Gates' method and found to be approximately 2 mm. To this heavy suspension enough normal sodium hydroxide solution was added to bring the reaction to pH 9.2, and it was then shaken for two hours and neutralized with normal hydrochloric acid solution, after which it was placed alternately in crushed solid carbon dioxide and in boiling water. In this manner it was frozen and thawed successively about thirty times. The freezing process was hastened by the addition of ether to the carbon dioxide. The smears of the suspension were examined at this time and the organisms appeared to be broken up and amorphous. In this process of autolysis of the bacterial cells, the suspension which was originally milky-white in color took on a yellowish tinge. It was passed through a Seitz filter. The filtrate was a clear, slightly amber-colored fluid. This was used in the intradermal tests, after first being tested for its primary toxicity. Along with it, pullorins *A, B,* and *C* were also tested to determine whether any false reaction might occur in apparently normal birds. These tests consisted in making intradermal tests with the pullorins and agglutination tests of nine normal cockerels. The results are given in table 3.

From table 3, it is seen that the toxicity tests failed to show that any of the pullorins used would produce a non-specific reaction and that the cockerels were negative to the agglutination test and to the bacteriological examination. Since none of the type *C* pullorins

TABLE 3

RESULTS OF TOXICITY TEST OF PULLORINS

Band No.	Type of pullorin used	Pullorin reaction	Agglutination test reactions				Post-mortem examination	Bacteriological findings
			1-25	1-50	1-100	1-200		
2826	A Concentrated	–	–	–	–	–	–	–
2827	B Precipitated	–	–	–	–	–	–	–
2817	C Cell-suspension	–	–	–	–	–	–	–
2800	density 1 cm	–	–	–	–	–	–	–
2815	C Cell-suspension density 2 cm	–	–	–	–	–	–	–
2813	C Cell-suspension density 3 cm	–	–	–	–	–	–	–
2810	C Cell-suspension density 4 cm	–	–	–	–	–	–	–
2831	D	–	–	–	–	–	–	–
2835	Cell-solution	–	–	–	–	–	–	–

caused a non-specific reaction, the one having the greatest density (1 cm) was selected for use in subsequent tests.

Preliminary Tests.—For these tests, artificially infected birds were used. Each bird of three groups of 17 cockerels received 1 cc each of live suspension of *Bacterium pullorum.* Group I was injected intraperitoneally, Group II intramuscularly, and Group III intravenously. Two weeks later, all the cockerels were bled for the agglutination test and were given the intradermal test with pullorins *A, B,* and *C.* The intradermal reactions were read after 24 hours. Following this, all the birds were killed and examined. The results are given in table 4.

Table 4 illustrates that while agglutinins were formed in the blood of some of the cockerels, the negative bacteriological and autopsy findings showed that there was no infection present. The failure of pullorins to cause an intradermal reaction in spite of the presence of agglutinins is in accordance with the contention of Baldwin and Krause as quoted by Fleischner, Meyer and Shaw[26] "that cutaneous hypersensitiveness is never present without a focus"; in other words, an anatomical foothold of infection is necessary to elicit a skin reaction.

TABLE 4

RESULTS OF PULLORIN AND AGGLUTINATION TESTS AND OF POST-MORTEM EXAMINATION OF ARTIFICIALLY-INFECTED COCKERELS

Mode of infection	Type of pullorin	Number of fowls tested	Results of pullorin test	Results of agglutination test	Macroscopic lesions present	*Bacterium pullorum* isolated	Number of fowls
Intramuscular	*A*	17	−	+	−	−	3
	Concentrated		−	−	−	−	14
Intraperitoneal	*B*	17	−	+	−	−	9
	Precipitated		−	−	−	−	8
Intravenous	*C*	17	−	+	−	−	10
	Cell-suspension		−	−	−	−	7

Pullorin Tests of Adult Hens.—Two hundred and fifty hens were used in this experiment. One hundred forty-six were White Leghorns which had been culled from a commercial flock because of non-productiveness and which had not previously been tested; 19—of which 17 were Rhode Island Reds and 2 White Leghorns—were reactors from a flock that had been previously tested by the agglutination method, and 85 were White Leghorns that had been used for experimental agglutination tests.

In making the intradermal tests, no attention was paid to the previous agglutination records. Hens were bled for agglutination tests simultaneously with the pullorin test. The summary of results obtained with different pullorins and records of agglutination tests with bacteriological findings are presented in table 5.

From the data given in table 5, it is seen that the results of the agglutination tests coincided closely with the bacteriological findings. Of the entire 250 birds tested and examined, disagreements were encountered only in the case of 3 bacteriologically positive birds that failed to react and of 2 bacteriologically negative birds that reacted to the agglutination test.

There was considerable variance in the accuracy of the results from the different pullorins. The concentrated pullorin detected only 4 (44.4 per cent) of the 9 bacteriologically positive birds and caused a reaction in 8 (13.6 per cent) of the 58 bacteriologically negative birds.

The precipitated pullorin failed to detect the 2 birds that were bacteriologically positive, and caused 10 (32.2 per cent) of the 31 bacteriologically negative birds to react.

The cell-suspension pullorin caused a reaction in 13 (92.8 per cent) of 14 bacteriologically positive birds and also in 22 (53.6 per cent) of 41 bacteriologically negative birds.

TABLE 5

COMPARATIVE RESULTS OF TESTS WITH CONCENTRATED, PRECIPITATED, CELL-SUSPENSION, AND CELL-SOLUTION PULLORINS AND BY THE AGGLUTINATION METHOD, AND OF THE POST-MORTEM EXAMINATION OF THE FOWLS TESTED

Type of pullorin	Number of fowls tested	Results of pullorin test	Results of agglutination test	Macroscopic lesions present	*Bacterium pullorum* isolated	Number of fowls	Per cent of fowls
A Concentrated	67	+[1]	+	+	+	4	5.9
		+[2]	−	−	−	8	11.9
		−	+	+	+	5	7.4
		−	−	−	−	50	74.6
B Precipitated	33	+[3]	−	−	−	10	30.3
		−	−	+[4]	−	2	6.0
		−	−	+	+	1	3.0
		−	+	+	+	1	3.0
		−	−	−	−	19	57.5
C Cell-suspension	65	+[5]	+	+	+	12	18.4
		+[6]	−	+	+	1	1.5
		+[7]	−	−	−	22	33.8
		−	+	+	+	1	1.5
		−	−	−	−	29	44.6
D Cell-solution	85	+[8]	+	+	+	14	16.4
		+[9]	−	+	+	1	1.1
		+[10]	−	−	−	1	1.1
		−	+	−	−	2	2.3
		−	−	−	−	67	78.8

[1] 3 reactions recorded as P_1, 1 as P_2.
[2] 6 reactions recorded as P_1, 2 as P_2.
[3] 9 reactions recorded as P_1, 1 as P_2.
[4] Abnormal yolks in both cases. *Bacillus coli* isolated from one, a *Coccus* from the other.
[5] 10 reactions recorded as P_1, 2 as P_2.
[6] Reaction recorded as P_1.
[7] 17 reactions recorded as P_1, 5 as P_2.
[8] 11 reactions recorded as P_1, 3 as P_2.
[9] Reaction recorded as P_1.
[10] Reaction recorded as P_1.

In contrast to the above, the cell-solution pullorin detected all of 15 bacteriologically positive birds and produced a reaction in only 1 (1.4 per cent) of 70 bacteriologically negative birds. The size of the pullorin reaction was recorded as P_1 in 8 fowls, and as P_2 in 3. While a more pronounced reaction would have facilitated the readings, it was possible, as the results show, to accurately detect and interpret these reactions.

In addition to the 85 birds just discussed, a flock of 143 fowls, consisting of White and Black Leghorns, Rhode Island Reds, Barred Rocks, Black Minorcas, and representatives of several other breeds, were tested with the cell-solution pullorin and by the agglutination method. These fowls were not available for post-mortem examination

TABLE 6

SUMMARY OF THE RESULTS OF PULLORIN AND AGGLUTINATION TESTS AND OF THE BACTERIOLOGICAL EXAMINATION OF 570 FOWLS

Group	Type of pullorin	Source of pullorin	Number of fowls tested	Bacteriologically positive fowls[1]								Bacteriologically negative fowls[2]						
				Total number	That were detected by				That were not detected by				Total number	That reacted to					
					Pullorin test		Agglutination test		Pullorin test		Agglutination test			Pullorin test			Agglutination test		
					Number	Per cent[3]	Number	Per cent[3]	Number	Per cent[3]	Number	Per cent[3]		Number	Per cent[4]	Number with gross lesions	Number	Per cent[4]	Number with gross lesions
1	Concentrated	Univ. of Calif.	67	9	4	44.4	9	100	5	55.5	0	0.0	58	8	13.8	0	0	0.0	.....
2	Precipitated	Commercial	85	70	52	74.2	70	100	18	25.7	0	0.0	15	11	73.3	5	3	20.0	2[5]
		Univ. of Illinois	235	97	88	90.7	96	98.9	9	9.2	1	1.0	138	44	31.8	1	4	2.9	1
		Univ. of Calif.	33	2	0	0.0	1	50	2	100.0	1	50.0	31	10	32.2	0	0	0.0	.....
3	Cell-suspension	Univ. of Calif.	65	14	13	92.8	13	92.8	1	7.1	1	7.1	51	22	43.1	0	0	0.0	.....
4	Cell-solution	Univ. of Calif.	85	15	15	100.0	14	93.3	0	0.0	1	6.6	70	1	1.4	0	2	2.8	0

[1] Fowls from which *Bacterium pullorum* was isolated. Gross lesions present in all.

[2] Fowls from which *Bacterium pullorum* was not isolated.

[3] Per cent of bacteriologically positive fowls.

[4] Per cent of bacteriologically negative fowls.

[5] These two birds are identical with two of the five bacteriologically negative fowls with gross lesions that reacted to pullorin.

and therefore data concerning them were not included in table 5. All gave a negative reaction to the agglutination test. One fowl, a Black Minorca (C-211), reacted to the pullorin test. The tests on this bird were repeated a month later with the same results. The bird was then secured for autopsy. The only abnormalities found were two small slightly blood-tinged yolks. *Bacterium pullorum* was not isolated. The results of the tests of these 143 negative birds are of value principally in indicating that the intradermal injection of the cell-solution pullorin is not liable to produce a non-specific or false reaction.

SUMMARY AND DISCUSSION

Table 6 gives a summary of the results of all of the pullorin and agglutination tests and of the post-mortem examination of the birds. A study of this table shows a very close correlation between the results of the agglutination tests and the bacteriological findings. A positive agglutination reaction was obtained with all but 4 (1.4 per cent) of the 207 birds from which *Bacterium pullorum* was isolated. This is a surprisingly high percentage of reactions to a single test of a group of infected fowls. Of the 363 bacteriologically negative fowls, all except 9 (2.4 per cent) gave a negative agglutination reaction. This can be regarded as an expected occurrence since the experience of numerous investigators has shown that non-infected fowls seldom react to the agglutination test. The positive agglutination reactions of these bacteriologically negative birds might have been due either to recovery and immunity of the birds, or to infection that was present, but which was not obtained in culture. This latter is particularly apt to be true of the 3 bacteriologically negative birds in which gross lesions were present.

In contrast to the results with the agglutination test, wide discrepancy is seen to exist between the bacteriological findings and the results of the pullorin tests, with the exception of those obtained with the cell-solution pullorin. This discrepancy consists, first, of the failure of from 7.1 per cent to 55.5 per cent of the bacteriologically positive birds in the various groups to react to the pullorin test; and, second, of a positive pullorin reaction in from 13.8 per cent to 73.3 per cent of the bacteriologically negative birds. Variations in these respects are seen in the results obtained from the concentrated, precipitated, and cell-suspension types of pullorin, but, in all instances, the degree of error is sufficient to make the results very unsatisfactory.

The reactions of the 85 birds that were tested with the cell-solution pullorin and later examined bacteriologically, however, are in remarkably close agreement with the bacteriological findings. Furthermore, the results of the test with this pullorin of the 143 birds, only one of which was examined bacteriologically, but all of which failed to react to the agglutination test, furnish evidence that this pullorin is not liable to cause a non-specific or false reaction in non-infected birds. These results, while encouraging, cannot be considered as conclusively indicating the true diagnostic value of the product because of the limited number of fowls on which it has been used.

CONCLUSIONS

The concentrated, alcohol-precipitated, and cell-suspension types of pullorin were not satisfactory preparations for use in the detection of carriers of *Bacterium pullorum.* The agglutination test is so much more accurate for this purpose than intradermal tests performed with these types of pullorin that the latter should be discarded until a better agent for use in making tests by the intradermal method can be perfected. The preliminary results from intradermal injections of the cell-solution type of pullorin indicate that this preparation is a promising agent worthy of further trial.

LITERATURE CITED

[1] JONES, F. S.
1912. The value of the macroscopic agglutination test in detecting fowls that are harboring *Bacterium pullorum*. New York State Vet. Col. Rpt. **1911–12**:149–158.

[2] BEACH, B. A., J. G. HALPIN, and C. E. LAMPMAN.
1927. Results of white diarrhea investigation. Jour. Amer. Vet. Med. Assoc. **70**:605–611.

[3] BEACH, J. R.
1927. Variation in the reactions obtained in repeated agglutination tests of the same fowls with *Bacterium pullorum* antigen. Hilgardia **2**:529–544.

[4] FITCH, C. P., and R. E. LUBBEHUSEN.
1928. The agglutination test as applied to bacillary white diarrhea. Cornell Vet. **18**:19–27.

[5] NEWSOM, I. E., F. CROSS, and O. C. UFFORD.
1928. On the accuracy of the agglutination test for *Bacterium pullorum* infection as shown by repeated tests on the same birds. Jour. Amer. Vet. Med. Assoc. **72**:611–617.

[6] WARD, A. R., and B. A. GALLAGHER.
1917. An intradermal test for *Bacterium pullorum* infection in fowls. U. S. Dept. Agr. Dept. Bul. **517**:1–15.

[7] SCHERAGO, M., and J. P. BENSON.
1919. Experiments on the intradermal test for *Bacterium pullorum*. Cornell Vet. **9**:111–117.

[8] BEACH, J. R.
1919. Bacillary white diarrhea. California Agr. Exp. Sta. Rpt. **1918–19**:86.

[9] FULLER, J. W.
1923. The intradermal test for detecting fowls carrying *Bacterium pullorum*. New York State Vet. Col. Rpt. **1922–23**:59–61.

[10] BRUNETT, E. L.
1925. Bacillary white diarrhea; fatal septicaemia of chicks. Cornell Vet. **15**:303–314.

[11] CERNAIANU, C.
1926. Sur la typhose aviare en Roumanie. Son diagnostic pratique par l'intradermo-reaction. Rec. Med. Vet. **102**:347–353.

[12] EDDINGTON, B. H.
1926. Ohio Agr. Exp. Sta. Rpt. **1925–26**:99.

[13] BROERMAN, A.
1927. Biological and medicinal agents for poultry. Jour. Amer. Vet. Med. Assoc. **70**:597–604.

[14] GRAHAM, R., and E. A. TUNNECLIFF.
1927. Studies in the diagnosis of bacillary white diarrhea. Jour. Amer. Vet. Med. Assoc. **70**:612–628.

[15] IDEM.
1927. Illinois Agr. Exp. Sta. Ann. Rpt. **1926–27**:127–132.

[16] EDWARDS, P. R., and F. E. HULL.
1927. A comparison of the agglutination test and the intradermal test in the detection of bacillary white diarrhea. Jour. Amer. Vet. Med. Assoc. **71**:590–599.

[17] GWATKIN, R. A.
1927. A comparison of the agglutination and pullorin tests for the detection of carriers of *S. pullora*. Ontario Vet. Col. Rpt. **1927**: 42–45.

[18] STAFSETH, H. J., and F. THORP, JR.
1928. Studies of the agglutination and pullorin tests for bacillary white diarrhea as to the efficiency of each in detecting carriers of *Salmonella pullorum* infection. Jour. Amer. Vet. Med. Assoc. **72**:745–756.

[19] BUSHNELL, L. D.
1928. Comparison of serologic and pullorin tests for bacillary white diarrhea. Jour. Inf. Dis. **43**:60–66.

[20] BUSHNELL, L. D., and C. A. BRANDLEY.
1929. A comparison of pullorin reaction and the agglutination test for bacillary white diarrhea. Jour. Amer. Vet. Med. Assoc. **74**:364–371.

[21] BUSHNELL, L. D., and C. A. BRANDLEY.
1929. Some experiments on the control of bacillary white diarrhea. Jour. Amer. Vet. Med. Assoc. **74**:444–453.

[22] BEACH, B. A.
1928. Bacillary white diarrhea. Vet. Med. **23**:339–342.

[23] OWEN, J. A.
1928. An experience with bacillary white diarrhea. The North Amer. Vet. **9**:42–43.

[24] MALLMAN, W. L.
1927. An improved antigen for the agglutination test in bacillary white diarrhea. Jour. Amer. Vet. Med. Assoc., **71**(n.s.24):600–606.

[25] SCHOENHOLZ, P., and K. F. MEYER.
1927. The purification of abortin. Jour. Inf. Dis., **40**:453–468.

[26] FLEISCHNER, E. C., K. F. MEYER, and E. B. SHAW.
1919. A resumé of some experimental studies on cutaneous hypersensitiveness. Amer. Jour. Dis. Children, **18**:577–590.

The titles of the Technical Papers of the California Agricultural Experiment Station, Nos. 1 to 20, which HILGARDIA replaces, and copies of which may be had on application to the Publication Secretary, Agricultural Experiment Station, Berkeley, are as follows:

1. The Removal of Sodium Carbonate from Soils, by Walter P. Kelley and Edward E. Thomas. January, 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.
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