THE EPIDEMIOLOGY OF FOWL CHOLERA

II. Biological Properties of P. avicida

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Plate 6

(Received for publication, October 25, 1929)

At the outset of the present studies of fowl cholera, attention was directed to the etiological agent, *Pasteurella avicida*.[1] The early investigations of this organism are chiefly of historical significance; reports of its biological characteristics, its range of variation and adaptability, and indeed, of the means for its exact identification are incomplete and contradictory.

The unsatisfactory state of this information can be emphasized by a brief summary of the literature. Following the early investigations of Perroncito (1), Toussaint (2), Pasteur (3), and Kitt (4), *P. avicida*, together with similar bacteria from other animal hosts, were placed together and designated the "Pasteurella" or "hemorrhagic septicemia" group (5). But later investigators disagreed as to the properties of these organisms. Thus, *P. avicida*, according to Schirop (6) and others (7–12), ferments dextrose, saccharose, and mannite without the production of gas. Kaupp and Dearstyne (13), on the other hand, found dextrose alone to be acidified; Tanaka (14), maltose, in addition to the above; Higgins (15), acid production on lactose litmus agar. Indol formation in peptone water was observed by all the investigators mentioned, save Kaupp and Dearstyne (13) and Lignieres (16). Theobald Smith and others (17–18) report the organism to be highly virulent for fowl and rabbits; Turner (19) regards this as a diagnostic criterion. Wide discrepancies in results of serological studies are indicated by the reports of Bushnell (20), which state that less than 50 per cent of the strains examined were capable of acting as antigens, while Fitch and Nelson (21), on the other hand, divided their strains into definite groups according to their agglutination in specific sera.

Such conflicting reports made necessary a further study of *P. avicida*. To this end, 209 strains were collected from autopsies on

[1] Synonyms: *Bacillus avisepticus*, *Pasteurella avium*, *Coccobacillus avicida*, *Bacillus bipolaris aviseptica*, and *Pasteurella cholerae gallinarum*.

recent cases of undoubted fowl cholera. Thirty-one of these strains came from a commercial poultry farm on Long Island (Paper IV), 172 from six scattered farms in New Jersey, 4 from poultry in Northern New York, and 2 from Kansas. One further strain, "Pa," obtained from Dr. Theobald Smith, came originally from a farm in Pennsylvania. These 210 strains were submitted to routine and special examinations, the results of which are contained in the following paragraphs.

General Characteristics

Morphology. All strains appeared as small, rounded, bipolar bacilli, decolorizing in Gram stain and were non-motile. The bacilli seemed relatively large in blood films from infected fowls, and in films stained by Wright's method (Fig. 1). Involution forms occurred in old cultures, small, faintly colored, coccoid forms predominating. Attempts to demonstrate capsules with special stains were not successful, but on numerous occasions the highly virulent, so-called "fluorescent" colony forms showed a clear peripheral zone resembling that of an unstained capsule.

Growth in Fluid Media. The bacilli grew well in infusion broth plus a trace of hemolyzed rabbit or chicken blood. Four hours after loop inoculation of the fluid, turbidity was perceptible; after fifteen hours, it was maximal. Pellicle formation appeared occasionally in 3–4 day cultures, but "granulation" and sedimentation did not occur. Asparagin synthetic media did not support growth; Dunham's peptone water gave feeble and retarded growth. Growth rate was enhanced, however, when unheated fresh chicken tissue was added to infusion broth.

Carbohydrate Fermentation and Indol Production. The carbohydrate media was made up of Dunham's peptone solution plus 0.5 per cent sugar and 1.0 per cent Andrade's indicator. This was tubed in 5 cc. quantities, together with a small, inverted tube to detect gas production. All strains acidified dextrose, saccharose, and mannite without the formation of gas. The acidity was slight and tended to decrease as artificial cultivation was prolonged. One exception to this behavior was noted in the case of twenty strains from a single flock which showed, in addition to the above, acid in xylose. All strains

gave a positive indol test when grown 2+ days in Dunham's peptone and examined by Ehrlich's method.

Colony Morphology. The cultures when grown on infusion agar plus a trace of hemolyzed blood showed three distinct forms of colony. Colony morphology was found later to be associated with other definite properties and came to be used as a means of classification. Hence, considerable attention was devoted to the behavior of these strains on solid media.

The first colony type was designated "fluorescent." It is relatively large and when seen by transmitted light, is opaque and fluorescent. By reflected light, it is greyish-white and moist (Fig. 2). Three strains from the Long Island farm, those from northern New York, the Kansas, and "Pa" cultures, all "epidemic" strains, formed fluorescent colonies.

The second colony type was called "blue." It is much smaller than the fluorescent form, relatively transparent, grey-blue, and without fluorescence (Fig. 2). All strains from the New Jersey flocks in which fowl cholera was endemic were of the "blue" colony type. This form occured also when the fluorescent type strains were grown for extended periods in artificial media or with specific antisera.

The third form of colony has been termed "intermediate," because of its partial resemblance to both the "fluorescent" and "blue" types. Under optimum conditions it may be described as similar to the "fluorescent" colony, but less opaque and less fluorescent. Colony appearance of the "intermediate" type is variable, however. When fifteen hours old, it seems typically "fluorescent;" at forty-eight hours, quite "blue." When colonies of this type are crowded, those at the periphery appear "fluorescent" at twenty-four hours, and the central ones "blue;" later, all become "blue." Frequently "ring" colonies are seen, composed of a "blue" core and "fluorescent" ring. The "intermediate" colony type strains were obtained from a single farm on Long Island during epidemic and post-epidemic periods.

The permanency of these colony types was tested on media, in sera, and in the animal body. When passed on solid media, "blue" colony forms remained "blue," "intermediates" varied as described in the preceding paragraph, but did not actually change to either of the other two types. The "fluorescent" type, on the other hand, did not

alter on blood agar, but, on infusion agar after eight passages, showed a mixture of "fluorescent" and "blue" colony forms. In fluid media the variations were similar, except that "fluorescent" types changed to "blue" more rapidly. Passage in antisera did not change the colony form of the "blue" nor "intermediate" type, but the "fluorescent" forms were replaced by "blue" colonies. Direct passage in fowls by intrathoracic injection failed to change the colony types. At present, therefore, it seems that "blue" colony types are permanent, and that "intermediate" forms as well, although varying in appearance, do not change their type. "Fluorescent" colony strains, on the other hand, growing under unfavorable circumstances, change to the "blue" colony form.

Acid Agglutination Zone. The behavior in acid buffer solutions of seventy strains was tested according to the method of Northrop and de Kruif (22). On centrifuging it was found that the "blue" colony type strains sedimented easily, whereas suspensions of the "fluorescent" colony strains were very stable. This difference was in accordance with the results of the acid agglutination tests (Table I) which showed a characteristic zone for each type. The "fluorescent" colony strains fluocculated at pH 2.4 or not at all. The "blue" colony strains, on the contrary, showed either a wide zone, pH 2.4–5.4, or a zone well toward the alkaline side. Flocculation of the "intermediate" colony forms was limited to a more narrow region toward the acid range of the scale, pH 2.4–4.0.

Serum Agglutination. The antigenic relationships of the 210 strains were studied by direct and reciprocal agglutination and absorption tests.

Antisera were prepared by injecting rabbits intravenously with washed saline suspensions of organisms taken from agar plates and preserved with 0.2 per cent phenol. The injections were given on five successive days, with doses increasing regularly from 0.1 to 0.5 cc. Two additional injections of 0.5 cc. were administered at five day intervals. After a final rest period of ten days, the animals were bled.

The first test was made with a "Pa" strain antiserum. Antigen-antibody mixtures were read after a three hour period in a water-bath at 56°. All but ten strains were agglutinated. These non-agglutinating cultures were either "fluorescent" or "intermediate" colony forms with narrow acid agglutination zones.

Further tests were made with the "fluorescent" and "intermediate" colony strains to determine if possible whether they were antigenically unrelated to the other cultures or combined with specific antibody and unable to flocculate. In the first place, it was found that "blue" colony variants from the "fluorescent" forms agglutinated to titre in the "Pa" serum. Again, antisera prepared from two "fluorescent" and two "intermediate" colony forms were ineffective toward their

TABLE I

Acid Agglutination of P. avicida

Colony type	Source	Range of pH	Number of strains
Fluorescent	Autopsy	No agglutination	2
"	"	2.4 only	1
Blue variant	Fluorescent	2.4–5.4	2
" "	"	2.4–4.6	1
Blue	Autopsy	2.4–5.4	18
"	"	2.4–5.0	1
"	"	3.0–5.0	22
"	"	3.0–5.4	5
Intermediate	"	2.4 only	2
"	"	2.4–3.0	1
"	"	2.4–3.2	2
"	"	2.4–3.5	1
"	"	2.4–4.2	1
"	"	3.0–4.0	3
"	"	3.0–4.2	7
"	"	3.2–3.5	1

homologous antigens, but brought about strong agglutination of the "Pa" strain. Finally, reciprocal absorption tests were made according to the method of Krumwiede (23) to determine whether the non-agglutinating strains could absorb agglutinins from heterologous sera and, conversely, whether the heterologous strains could absorb agglutinins from the sera of the non-agglutinating strains. The results of three such tests are given in Table II. Strains 631 and 638 are representative of the "fluorescent," non-agglutinating form; Strain 728 is an example of the "blue" agglutinating form; Strain

TABLE II

Reciprocal Absorption Tests with P. avicida

Serum	Antigen	Result
	"Pa" and 728	
"Pa"—unabsorbed	"Pa"	Agglutination 1:320
" "	728	" 1:320
" —absorbed with "Pa"	"Pa"	No agglutination.
" " " "	728	" "
" " " 728	"Pa"	" "
" " " "	728	" "
728 —unabsorbed	"Pa"	Agglutination 1:320
" "	728	" 1:320
" —absorbed with "Pa"	"Pa"	No agglutination.
" " " "	728	" "
" " " 728	"Pa"	" "
" " " "	728	" "
	"Pa" and 631	
"Pa"—unabsorbed	"Pa"	Agglutination 1:320
" "	631	No agglutination.
" —absorbed with "Pa"	"Pa"	" "
" " " "	631	" "
" " " 631	"Pa"	" "
" " " "	631	" "
631 —unabsorbed	"Pa"	Agglutination 1:1280
" "	631	No agglutination.
" —absorbed with "Pa"	"Pa"	" "
" " " "	631	" "
" " " 631	"Pa"	" "
" " " "	631	" "
	"Pa" and 638	
"Pa"—unabsorbed	"Pa"	Agglutination 1:320
" "	638	No agglutination.
" —absorbed with "Pa"	"Pa"	" "
" " " "	638	" "
" " " 638	"Pa"	" "
" " " "	638	" "
638 —unabsorbed	"Pa"	Agglutination 1:600
" "	638	No agglutination.
" —absorbed with "Pa"	"Pa"	" "
" " " "	638	" "
" " " 638	"Pa"	" "
" " " "	638	" "

"Pa" an "intermediate" agglutinating form. Strains 631 and 638 did not agglutinate in homologous or heterologous sera; however, they did absorb agglutinins from homologous and heterologous sera. Furthermore, antibodies in sera from these non-agglutinating strains were absorbed by the homologous and heterologous strains. The test with the "blue" form 728 is included as a control, showing the complete absorption of agglutinating strains.

The antigenic relationship of chicken and rabbit strains of Pasteurellae was tested by titrating a) rabbit cultures, Rivers D and DC 30 (29) in chicken strain antisera 638 and "Pa," and b) fifteen fowl cholera strains, representing "blue," "fluorescent," and "intermediate" colony forms in rabbit strain, Rivers D, antiserum. No agglutination occurred, indicating that the fowl cholera strains differ antigenically from the rabbit strains tested.

Complement Fixation. To test further the antigenic relationship of strains of *P. avicida*, the complement fixation reaction was employed. Thirty strains were examined for their complement fixing power with "Pa" serum and a lesser number with sera from non-agglutinating strains previously described.

In general, the method described by Bull (24) was followed. The organisms to be used as antigens were well washed and suspended in salt solution. Their anti-complimentary titre was determined by finding the amount of suspension that would destroy the complement in 0.2 cc. pooled guinea pig serum diluted 1:10. The mixture of complement and bacteria was incubated at 37° for one hour, then tested with anti-sheep cell hemolytic system. One-third of the anti-complimentary titre was used as the antigenic dose in the final test. This amount of suspension was mixed with 0.5 cc. of the "Pa" serum, used in dilutions from 1:16 to 1:1200. Two units of complement were added, the tubes brought to a uniform volume of 1.5 cc., allowed to incubate at 37° for one hour, then left overnight in the icebox. The hemolytic system was added and incubated for one hour at 37°. Tubes in which hemolysis was entirely inhibited were considered positive.

The results of these complement fixation tests are given in Table III and compared with corresponding agglutination titres. The titre of various strains with other sera is presented in Table IV. The inagglutinable "fluorescent" colony strains proved fairly reactive in their ability to fix complement, and the "Pa" antigen fixed complement in various sera to the same extent as the homologous strains.

TABLE III

Results of Complement Fixation and Agglutination Tests

	Number of strains	Maximum effective dilution of "Pa" serum	
		Agglutination	Complement fixation
Fluorescent form	1	0	25
	1	0	256
	1	0	40
Blue form	1	1280	160
	1	1280	320
	2	2500	320
	2	2500	160
	2	2500	512
	1	2500	640
Intermediate form	2	0	256
	1	40	20
	1	50	40
	1	80	25
	2	80	50
	1	80	75
	1	160	40
	4	160	50
	1	160	160
	1	320	50
	3	640	640

TABLE IV

Complement Fixation Tests with Organisms of Different Colony Forms

Antigens	Maximum serum dilution showing complete fixation				
	Sera				
	"Pa"	770	773	629	638
"Pa"	50	75	200	300	900
770 ("Blue")	75	25	100	50	200
773 ("Blue")	50	100	200	100	200
629 ("Fluorescent")	40	50	100	100	600
638 ("Fluorescent")	20	25	75	100	600

These results are regarded as further evidence of the antigenic similarity of strains of *P. avicida*.

Toxin Formation. Numerous unsuccessful attempts were made to demonstrate the presence of a toxin resembling that described by Pasteur (25).

Virulent bacilli were grown in infusion broth, with and without the addition of hemolyzed blood and fresh, unheated chicken tissue. The cultures were incubated aerobically and anaerobically for periods of eight hours to one week. The filtrates were tested by intravenous, intramuscular, and subcutaneous injections into chickens. Some filtrates were concentrated to one-tenth volume by distillation *in vacuo* and then tested. A further series of inoculations was made with bacilli dissolved in dilute alkali. In no instance did the inoculated animals die, nor symptoms develop resembling those of fowl cholera.

Virulence. From an epidemiological point of view, no property of bacteria is more important than that of virulence or pathogenicity. Hence in these studies an effort has been made to determine with care the disease-producing power of *P. avicida.*

The test animals were chickens from a single flock of healthy breeding hens, shipped to this laboratory one day after hatching and raised under uniform conditions. Twenty birds, four weeks old, weighing 100–150 gms., were used for each test. The bacterial cultures for testing were grown on hemolyzed blood agar for 15–18 hours, washed off, and suspended in infusion broth. The density of the suspension was standardized with the Gates' turbidometer (26) and brought to the concentration of an 18 hour culture. The dose employed was 0.2 cc., controlled by counting, and representing about 20,000,000 bacilli. The inoculum was deposited from a syringe fitted with a blunt pointed needle, on the intact nasal mucosa. The reasons for using the intranasal portal of entry are discussed in the following paper. After inoculation, each bird was kept in a separate cage. The tests extended over a ten day period, although deaths were infrequent after the fourth day.

To control the effect of variations in susceptibility of the chickens, one strain of "fluorescent" colony type organisms was reinjected into a control group of twenty birds with each test.

The results of these titrations are given in Table V, where "virulence" is expressed as percentage of birds dying of fowl cholera. Other determinations, if made on the identical culture, are included. The data indicate a distinct correlation between virulence and the attributes associated with colony form. Thus, the "blue" colony types proved relatively avirulent, while the "fluorescent" forms were highly pathogenic. Single tests on the "intermediate" colony strains gave

results varying from 0 to 60 per cent mortality. For this irregularity, three explanations are possible,—technical difficulties, variations in the resistance of the birds, or actual differences in the virulence of the "intermediate" strains. Data to be presented in the succeeding

TABLE V

Virulence and Other Properties of Pasteurella avicida of Different Colony Forms

Colony form	Source	Strain	Acid agglutination (pH range)	Serum agglutination (Maximum dilution)	Virulence (Per cent mortality)
Fluorescent	Autopsy	629	2.4 only	0	10
		631	none	0	60 (av. 4)
		638	none	0	30 (av. 5)
Blue variant	Fluorescent	629 B	2.4–5.4	1280	0
		631 B	2.4–4.6	2500	0
		638 B	2.4–5.4	1280	0
Intermediate	Autopsy	729	3.0–4.2	2500	0
		730	3.0–4.2	2500	0
		731	3.0–4.2	1280	0
		733	2.4–3.5	320	0
		726	2.5–4.1	100	10
		798	2.5 only	0	0
		796	2.5–4.7	640	0
		635	3.0–5.4	1250	20
		641	2.4–5.0	1280	20
		642	2.4–5.4	640	60
		644	2.4–5.0	2500	20
		648	3.0–5.0	2500	40
Blue	Autopsy	651	3.0–5.0	5000	0
		647	2.4–5.0	640	0
		702	2.4–5.0	640	0
		773	2.4–5.4	5000	10
		779	2.5–5.2	640	0
		793	2.5–5.2	1250	0
		781	2.5–5.2	1250	20

papers render the latter possibility unlikely and indicate that strains of a given colony type are in general of similar virulence. The relative pathogenicity of "fluorescent," "intermediate," and "blue" colony types may be expressed for convenience as 50 per cent, 25 per cent, and 0 per cent respectively.

Attempts to raise virulence by growth in media, sera, and passage through fowls were not successful. "Blue" colony forms could not be changed to "intermediate" or "fluorescent," nor could an increase in virulence of any strain be demonstrated. Tests by means of host passage were made by intranasal instillations with and without the use of brilliant green (Bull, 27). One test at a season when titration mortality was low was carried on for seven passages (Table VI). The strain passed directly in blood from bird to bird showed no increase in virulence.

TABLE VI

Attempt to Increase Virulence by Chicken Passage

Passage	Preliminary treatment of chicken	Survival time in days	Form of organism recovered	Virulence
I	Brilliant green intranasally	2	"Fluorescent"	
II	" "	12	"	
III	None	6	"	
IV	"	6	"	
V	"	3	"	
VI	"	3	"	5 chicks inoculated, 1 died.
VII	"			5 chicks inoculated, none died.

DISCUSSION

This study of 210 freshly isolated strains from known cases of fowl cholera has proved useful in defining and classifying the inciting microbe of fowl cholera, *P. avicida*. This definition and classification were made on the basis of general uniformity in morphology and growth characteristics in fluid media and like reactions in carbohydrates and indol production. By the aid of serological methods, a fundamental antigenic similarity of all strains of *P. avicida* has been determined. The extent of this specificity is, however, unknown. It may indicate antigenic identity of all strains or the presence of a common antigenic fraction among otherwise distinct serologic groups similar to the "protein" fraction of pneumococci (28). At present, however, further analysis is hindered by the apparent inagglutinability of certain otherwise typical strains.

However, a temporary classification of *P. avicida* is suggested on the basis of colony morphology. One type, "fluorescent," is associated with epidemics of fowl cholera, is highly virulent, stable in suspension, agglutinable only in very acid buffers, pH 2.4, and not at all in antisera. It resembles the "D" form of *P. lepiseptica* (29). The second type, "blue," is found in flocks where fowl cholera is endemic, is of low virulence, unstable in suspension, agglutinates in acid buffers over a wide zone, pH 2.4 – 5.6+, and in all *P. avicida* antisera. This type corresponds to the "G" form of *P. lepiseptica* (29). The third type, "intermediate," is associated with the more severe fowl cholera, and is "intermediate" in its behavior. This form bears some resemblance to the mucoid types of *P. lepiseptica* (29). These groupings have remained consistent throughout the present studies.

Hence there is available a definite criterion for the diagnosis of *P. avicida*, together with a sub-grouping of considerable epidemiological importance.

SUMMARY

1. A bacteriological study has been made of 210 fresh strains of Pasteurella obtained from typical cases of fowl cholera on seven widely separated poultry farms.

2. The strains have proved identical in consisting of small, pleomorphic, bipolar staining, Gram-negative, non-motile bacilli. They grew rapidly in infusion broth plus a trace of hemolgobin. They formed acid but no gas in media containing dextrose, saccharose, and mannite; indol was produced.

3. The strains fall into three distinct groups, according to their colony formation on hemoglobin agar. The "fluorescent" colony was large, whitish, opaque, exhibiting, under suitable conditions marked fluorescence. The "blue" colony was smaller, clear slate-blue, and non-fluorescent. The "intermediate" colony was moderately fluorescent at 15–18 hours growth, and "blue" thereafter. It was "blue" at all times when crowded and occasionally of "ring" form. "Fluorescent" colony cultures developed "blue" colony forms under certain conditions; otherwise all forms were stable.

4. Strains from "fluorescent" colonies were resistant to precipitation by acids, to sedimentation by centrifuging, and although they com-

bined with specific antiserum, did not agglutinate. They were relatively highly virulent and occurred in flocks where fowl cholera was epidemic.

5. Strains from "blue" colonies were precipitated by acids over a wide range of concentration and agglutinated strongly in antisera. They were relatively of low virulence and occurred in flocks where fowl cholera was endemic.

6. Strains from "intermediate" colonies varied in behavior betweeen the "fluorescent" and "blue" strains. They came from a flock where fowl cholera was epidemic.

BIBLIOGRAPHY

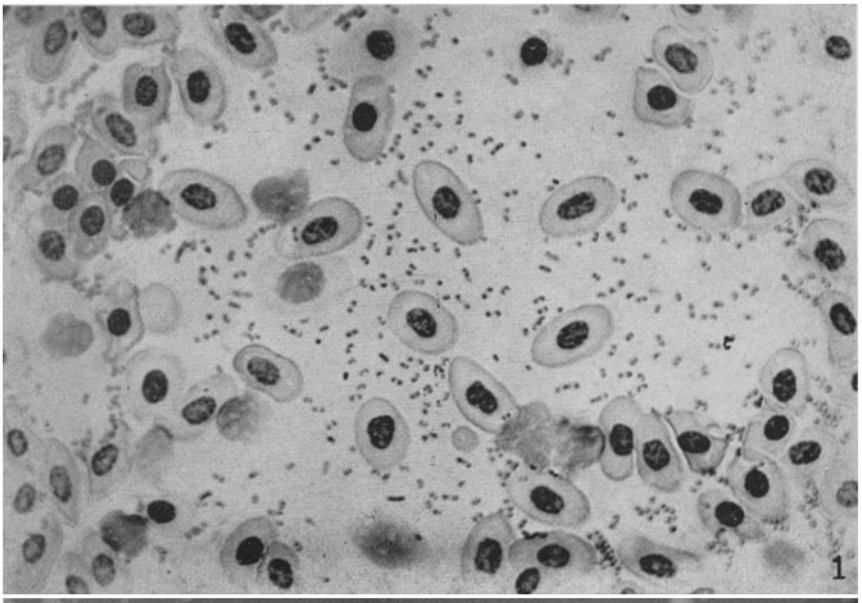
1. Perroncito, E., *Arch. f. Tierheilk.*, 1879, **5**, 22.
2. Toussaint, H., *Rec. d. med. vet.*, 1879, p. 946.
3. Pasteur, L., *Compt. rend. Acad. Sci.*, 1880, **90**, 239, 952, 1030; *ibid.*, 1880, **91**, 673.
4. Kitt, T., *Mh. Tierheilk.*, 1893, **4**, 59; *ibid.*, 1897, **8**, 529.
5. Hueppe, F., *Berlin. klin. Wchnschr.*, 1886, **23**, 753.
6. Schirop, H., *Z. f. Bakt., Orig.*, 1908, **47**, 307.
7. Vorloud, *Z. f. Bakt., Orig.*, 1908, **45**, 97, 193.
8. Mack, W., and Records, E., *Ag. Exp. Sta., Nevada State Coll. Bull., No. 85*, 1916.
9. Bushnell, L., and Beaudette, F. R., *J. Am. Vet. Med. Assn.*, 1922, **13**, 729.
10. Fitch, C., and Nelson, E., *J. Am. Vet. Med. Assn.*, 1923, **16**, 147.
11. Patton, J., *J. Am. Med. Assn.*, 1926, **21**, 581.
12. Manninger, R., *Z. f. Bakt., Orig.*, 1919, **83**, 525.
13. Kaupp, B., and Dearstyne, R., *J. Am. Vet. Med. Assn.*, 1925, **20**, 249.
14. Tanaka, A., *J. Inf. Dis.*, 1926, **38**, 421.
15. Higgins, C. H., *J. Exp. Med.*, 1898, **3**, 650.
16. Lignieres, J., *Bull. Soc. centr. d. Med. vet.*, 1900, **18**, 329.
17. Harvey, W., and Iyengar, K., *Indian J. Med. Res.*, 1922, **9**, 726.
18. Hadley, P., *Ag. Exp. Sta., Rhode Island State Coll. Bull., No. 159*, 1914; *Z. f. Bakt., Orig.*, 1913, **69**, 271.
19. Turner, A., *J. Australian Vet. Assn.*, 1926, **2**, 127.
20. Bushnell, L., *Proc. Soc. Am. Bact., Abs. Bact.*, 1923, **7**, 30.
21. Fitch, F. and Nelson, E., *J. Am. Vet. Med. Assn.*, 1923, **16**, 147.
22. Northrup, J. H., and de Kruif, P. H., *J. Gen. Physiol.*, 1922, **4**, 639.
23. Krumwiede, C., Cooper, G., Provost, D., *J. Immunol.*, 1925, **10**, 55.
24. Bull, C., (Personal communication).
25. Pasteur, L., *Compt. rend. Acad. Sci.*, 1880, **90**, 1030.
26. Gates, F. L., *J. Exp. Med.*, 1920, **31**, 105.
27. Bull, C., *Proc. Soc. Exp. Biol. and Med.*, 1926, **24**, 183.

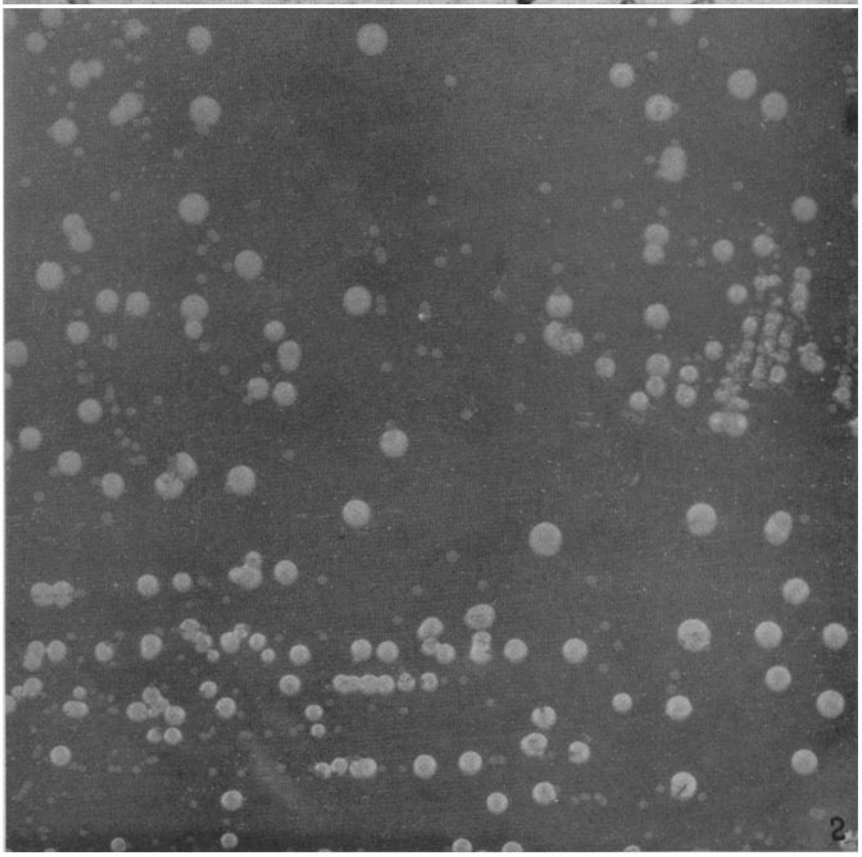
28. Avery, O., and Heidelberger, M., *J. Exp. Med.*, 1923, **38**, 81.
29. Webster, L. T., and Burn, C. G., *J. Exp. Med.*, 1926, **44**, 343.

EXPLANATION OF PLATE 6

FIG. 1. *P. avicida* in blood of experimentally infected fowl. Antemortem specimen.

FIG. 2. "Fluorescent" and "blue" colony forms of *P. avicida*.





(Hughes: Epidemiology of fowl cholera. II)