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Characteristics of *Pasteurella multocida* of Human Origin

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Physiological, serological, morphological, and cultural differences were observed among 30 *Pasteurella multocida* cultures of human origin. The usual variations in the fermentation of glycerol, lactose, sorbitol, trehalose, and xylose were observed. Unlike most *P. multocida*, two cultures did not produce indol. Six serotypes were found. In addition to the widely recognized iridescent, blue, and watery mucoid (circular) colonies, punctiform colonies were observed. None of the cultures were pathogenic for turkeys. Results of the study indicate that one should be aware of the many variable characteristics of *P. multocida* of human origin to facilitate indentification.

Pasteurella multocida was first isolated by M. Toussaint in 1879 from a chicken (18, 27). Since then, *P. multocida* has been isolated from many species of mammals (1, 22) and birds (7, 23). It has been isolated from many regions of the body of humans (12, 14, 15), mostly from the upper respiratory tract and from wounds inflicted by dogs and cats. Probably many cultures of human origin are unreported or unidentified. Significant morphological, physiological, and serological variations exist among isolants of *P. multocida;* these variations may lead to confusion in identification but may aid in epidemiological or epizootical studies. However, none of these characteristics will determine the host species of origin or the virulence of the organism. Because humans are often infected with *P. multocida*, they may be a source of infection for livestock and poultry.

The purpose of these studies was to compare the morphological, physiological, serological, and growth characteristics of 30 *P. multocida* cultures of human origin with those we have observed from avian and mammalian species and to determine whether those cultures were pathogenic for turkeys. Turkeys are highly susceptible to avian strains of *P. multocida* (7).

MATERIALS AND METHODS

Twenty-seven cultures of *P. multocida* (Table 1) were received from D. P. Nicholson, University of Iowa Hospital, Iowa City. One culture (Table 1, laceration) was received from F. C. Blank, Wilmington Medical Center, Wilmington, Del., and two cultures (Table 1, dog bite wounds) were received from D. Hansman, Adelaide Children's Hospital, North Adelaide, SA, Australia. Cultures were lyophilized after they were received and reconstituted when needed.

Cultures were streaked on dextrose starch agar (Baltimore Biological Laboratories, Cockeysville, Md.) and examined after 17 to 24 h at 37 C with a stereomicroscope illuminated by obliquely transmitted light (7). The colonies were recorded as fluorescent (iridescent) or blue (3, 16) and watery mucoid (28). Blood agar plates (blood agar base, Difco, containing blood) were examined for hemolysis after 24 h at 37 C and 48 h at 23 C. The ability to grow on MacConkey agar (Difco, Detroit, Mich.) was determined at 37 C for 48 h and then at 23 C for 48 h. Motility was determined in semisolid motility medium (Difco) after 48 h at 37 C and after 12 days at 23 C. Cellular morphology was observed after cells from a colony were stained with Wright and Gram stains. Capsules were observed by mixing a drop of 0.85% NaCl solution containing cells from various colony types with a drop of india ink on a glass slide, covering with a glass cover slip, and examining the preparation with an oil immersion objective.

Fermentation reactions were determined in phenol red broth base (Difco) containing 1% of the various compounds and sterilized by filtration. Results were recorded after 24 and 48 h at 37 C and after 12 days at 23 C. Hydrogen sulfide (H_2S) production was tested by suspending strips of filter paper impregnated with lead acetate above the broth cultures (11). Results were recorded after 24 and 48 h at 37 C and 12 days at 23 C. Solid media usually used to detect the production of H_2S by enteric bacteria were not satisfactory for testing *P. multocida* (9). Production of indol was tested after 24 h at 37 C in broth consisting of 2% tryptose (Difco)-0.85% NaCl (pH 7.2) by using a modified Kovac method (5); negative cultures were retested daily for 5 days. Urease production was determined after 48 h at 37 C and after 12 days at 23 C in urea broth (Difco). The tests for cytochrome oxidase, catalase, and methyl red were described previously (29).

The gel diffusion precipitin test was used to serotype the cultures (8). Antisera were prepared with water-in-oil emulsified antigens that were injected

TABLE 1. *Serotypes and physiological characteristics that differed among 30 Pasteurella multocida cultures of human origin*

Specimen		Serotype	Fermentation					Indol production
Source	Quantity		Glycerol[a]	Lactose	Sorbitol	Trehalose	Xylose	
Bronchial aspiration	1	3	−	−	+	−	+	+
Bronchial aspiration	1	4	+	−	+	−	+	+
Cat bite	1	3	+	−	+	−	+	+
Dog bite	1	3	+	−	+	−	+	+
Dog bite	1	—	+	−	+	−	+	+
Laceration	1	1[b]	+	+	+	−	+	+
Sacral ulcer	1	3-4	+	−	+	−	−	+
Sinus washing	1	3	−	−	+	+	+	+
Sinus washing	3	3	+	−	+	−	+	+
Sinus washing	1	4	+	−	+	+	+	−
Sinus washing	1	4	+	−	+	+	+	+
Sinus washing	2	4	+	−	+	−	+	+
Sinus washing	1	3	−	−	+	−	+	+
Sputum	1	3	−	−	+	+	+	−
Sputum	8	3	+	−	+	−	+	+
Sputum	1	6	+	−	+	−	−	+
Sputum	1	4	+	−	+	+	+	+
Sputum	1	4	+	−	+	−	+	+
Peritoneal swab	1	13	−	−	−	+	+	+
Throat swab	1	12	−	−	+	−	−	+

[a] Usually fermented by second or third day.
[b] Culture did not react with typing antisera, but antiserum prepared with this culture reacted with types 3, 6, and 7 antigens.

subcutaneously in the neck of mature male New Hampshire chickens. The birds were exsanguinated 3 weeks after injection. Feed was removed from cages the night before they were exsanguinated. The serum was separated from the clot within 3 h by centrifuging 1 h at 1,500 $\times$ *g* and preserved with 0.01% thimerosal and 0.06% phenol. Antigens were prepared with 18- to 24-h growth from a heavily seeded culture plate (dextrose starch agar) suspended in 1.0 ml of 8.5% NaCl, 0.02 M phosphate, and 0.3% formalin solution (pH 7.0). The cell suspension was heated in a water bath at 100 C for 1 h and sedimented by centrifugation, and the supernatant was used as the antigen. The gel consisted of 0.9% Special Noble agar (Difco)-8.5% NaCl in distilled water. Melted agar (5 ml) was placed on microscope slides (25 by 75 mm); wells, 4 mm in diameter and 6 mm from center to center, were cut as shown in Fig. 3. Results were recorded after 24 to 48 h at 37 C.

Pathogenicity was determined in 5-week-old Beltsville White turkeys (National Animal Disease Laboratory flock) by swabbing the nasopharynx with a cotton swab saturated with a bacterial suspension (10). Three turkeys were used for each culture. The bacterial suspensions were prepared by reconstituting the lyophilized cultures in tryptose broth (Difco) and streaking on dextrose starch agar plates. After 24 h at 37 C, colonies were suspended in tryptose broth to a density of 80% light transmission at 600 nm as determined with a Coleman Junior spectrophotometer (Model 6-A). The above density is equivalent to approximately one billion organisms per milliliter. Each bird received approximately 1.1×10^8 organisms per dose. The turkeys were observed for 3 weeks after exposure to the cultures.

RESULTS

Colonies from the 30 cultures on dextrose starch agar varied in size from 0.1 to 2.5 mm in diameter (Fig. 1). Nine of the cultures produced watery mucoid colonies (Fig. 1A and 2B), five cultures produced iridescent colonies, ten produced blue colonies, and three produced a mixture of iridescent and blue colonies (Fig. 1B and 2A). Three cultures produced iridescent colonies with sectoring at the edge (7). Typical iridescent, blue, and water mucoid colonies are shown in Fig. 2. Colonies were smooth and entire, but occasionally punctiform colonies were concentrically ringed or had the appearance of cut glass. Colonies on blood agar were smaller and opaque when examined with reflected light (Fig. 3). The consistency of the colonies was butyrous, except for the watery mucoid colonies, which were viscous. On agar slants, the growth from watery mucoid colonies gravitated to the bottom of the tubes. After 24 to 48 h colonies were larger and began to lose their distinguishing characteristics when observed with obliquely transmitted light. The cultures on agar produced a decided odor.

All cultures were gram negative. The bacterial cells of the various isolants ranged from 0.6 to 5.0 μm in length, as measured with a Bausch and Lomb filar micrometer eye piece. They occurred singularly and occasionally in pairs or chains of up to 20 cells. Many cells stained bipolar, but no culture produced cells that were all distinctively stained bipolar, as usually seen in cultures from animals with a septicemia (7). Biopolar staining was best demonstrated with Wright stain. Halos that indicate the presence of a capsule were observed around cells from watery mucoid and iridescent colonies but not from blue colonies.

The fermentation of glycerol, lactose, sorbitol, trehalose, and xylose and the production of indol were variable (Table 1). Characteristics that were common to all isolants are listed in Table 2.

Serotypes 1, 3, 4, 6, 12, and 13 were observed (Table 1). One culture reacted with equal intensity with types 3 and 4 antisera (Fig. 4). Serotypes 12 and 13 were new serotype (Fig. 4). Four of the type 3 cultures also reacted slightly with type 4 antiserum, and four type 4 cultures also reacted slightly with type 7 antiserum.

No turkeys died, and no signs of infection were observed.

DISCUSSION

The *P. multocida* isolated from the respiratory tract of humans may or may not have been responsible for disease, because some healthy animals carry *P. multocida* (17, 20, 24). None of the cultures were pathogenic for turkeys. They probably would have been pathogenic for mice and rabbits because these animals are highly susceptible to most strains of *P. multocida*, regardless of the host from which they are isolated. Virulence of *P. multocida* is dependent on the strain, the host species, within-strain and host variations, and condition of contact between the host and bacterium. Healthy cattle are often carriers of *P. multocida*, but they may develop pneumonia after they are predisposed to a virus or adverse environmental conditions (20). If *P. multocida* causes disease of the respiratory tract of humans, it probably depends more on a change in the normal resist-

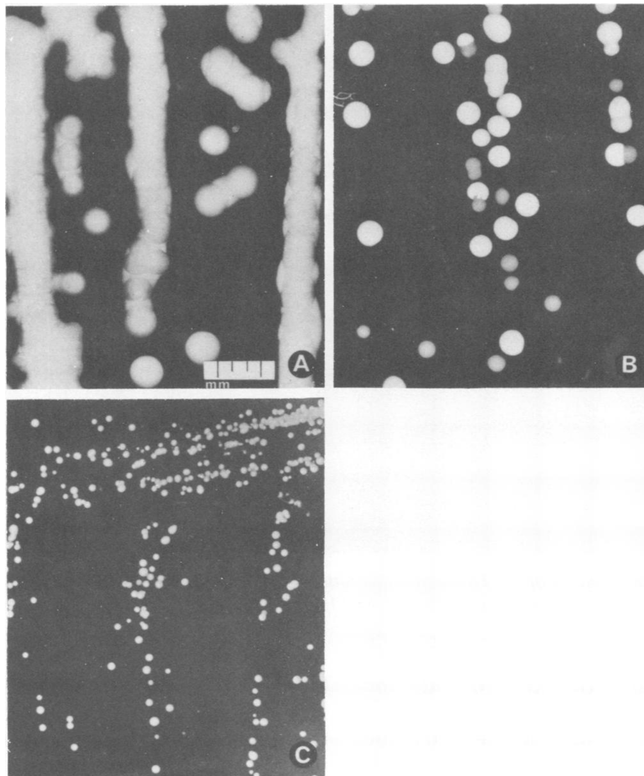

FIG. 1. *Comparative size of 18-h colonies on surface of dextrose starch agar illuminated with oblique light. (A) Watery mucoid; (B) mixture of iridescent and blue (dark); (C) iridescent, punctiform.*

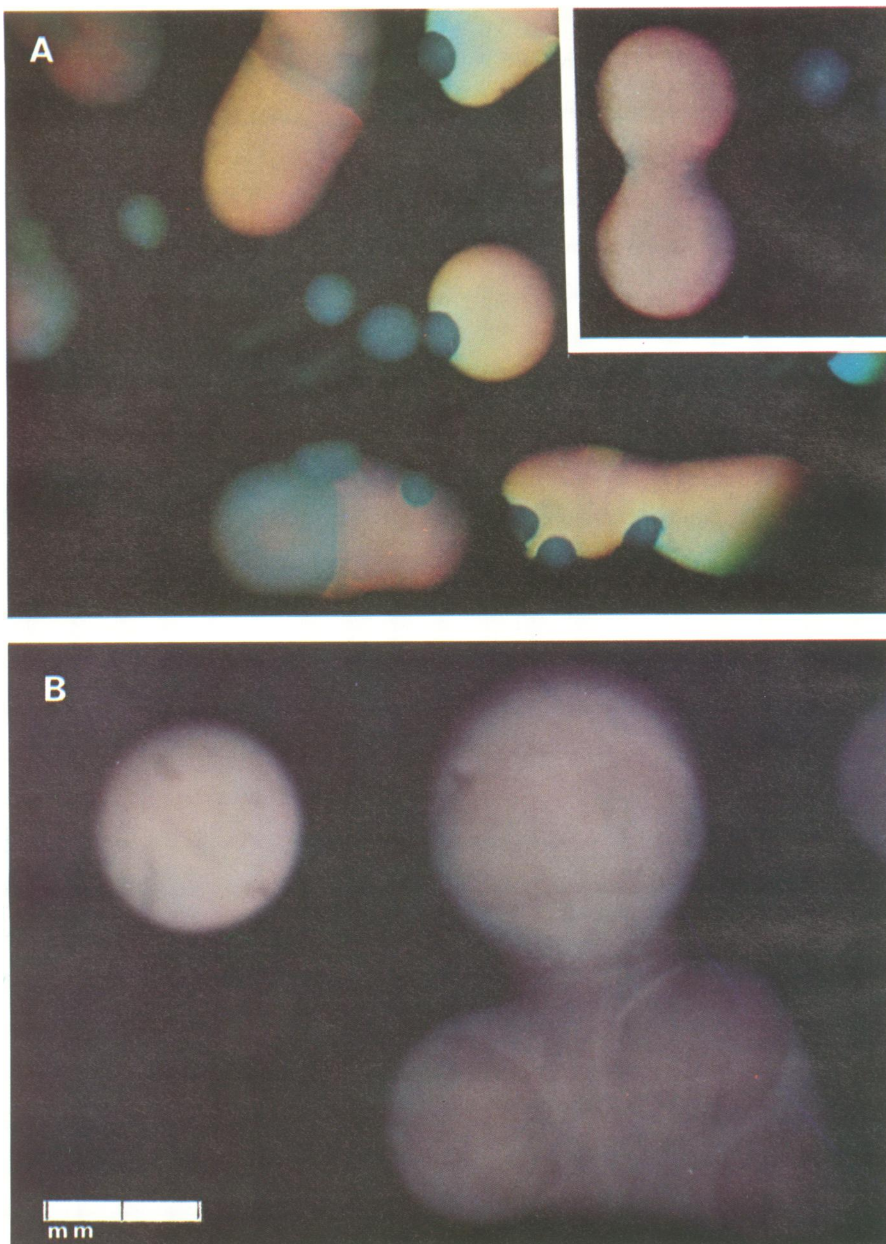

FIG. 2. *Optical characteristics of the three most prevalent colony types of P. multocida on surface of dextrose starch agar illuminated by oblique transmitted light (18-h colonies). (A) Mixture of iridescent and blue; (B) watery mucoid.*

ance of the host tissue rather than on inherent characteristics of the organism.

There is no absolute set of characteristics that can be used to identify all cultures of *P. multocida*, but their usual characteristics, regardless of the host from which they are isolated, are the fermentation of glucose, sucrose, mannitol, and fructose without producing gas, the nonfermentation of lactose, maltose, inositol, inulin, rhamnose, and salicin, the production of indol, the lack of urease, and the inability to hemolyze blood or grow on MacCon-

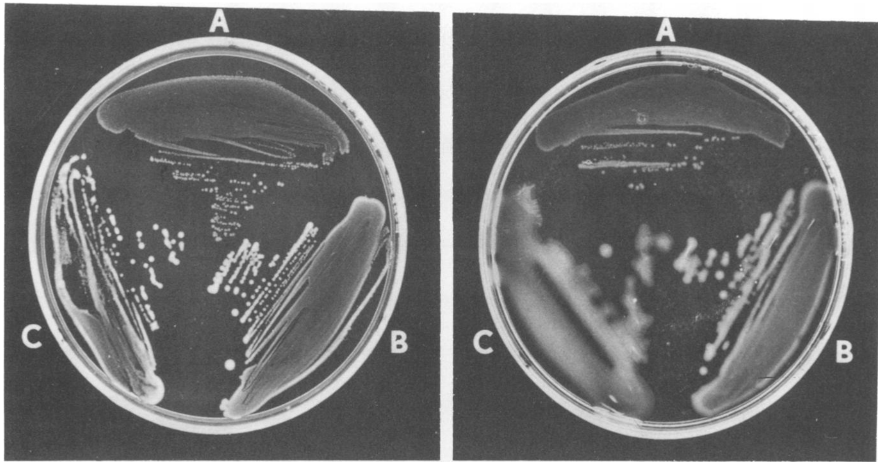

FIG. 3. *Blue (A), iridescent (B), and watery mucoid (C) P. multocida colonies on blood agar (left) and dextrose starch agar (right) illuminated with reflected light (18-h colonies).*

key agar. For comparative purposes, the results of various tests at this laboratory with over 1,000 cultures from cases of pasteurellosis other than human are presented in Table 3.

Holmes and Brandon (13) observed variations in the ability of *P. multocida* from humans to ferment lactose, mannitol, sorbitol, arabinose, dulcitol, and xylose. Smith (25) observed variations of isolants from humans to ferment xylose, sorbitol, mannitol, maltose, and trehalose. Talbot and Sneath (26) reported that an isolant from a wound inflicted by a dog produced acid and gas from sucrose, maltose, mannose, and trehalose; they made no reference as to its ability to produce urease. Rogers and Elder (21) reported that *P. multocida* from a dog produced acid and gas from glucose. They reported that urease was not produced, but in our laboratory their culture produced urease after 7 days of incubation. Gump and Holden (6) studied an isolant from an animal attendant with an infected cat bite wound, which they designated as a new species of *Pasteurella*. This culture produced urease and acid and gas from glucose, sucrose, and maltose. They stated that their culture "possesses a urease, but this is a variable characteristic of *P. multocida* and cannot be used to differentiate *P. new species* from *P. multocida*." We have never observed an isolant of *P. multocida* that produced urease. It would appear that further study is needed with the gas-producing *P. multocida*.

TABLE 2. *Physiological characteristics that were common to all 30 P. multocida cultures of human origin*

Test	Results
Arabinose	Not fermented
Dextrin	Not fermented
Dulcitol	Not fermented
Inositol	Not fermented
Inulin	Not fermented
Maltose	Not fermented
Raffinose	Not fermented
Rhamnose	Not fermented
Salicin	Not fermented
Galactose	Fermented without gas
Glucose	Fermented without gas
Fructose	Fermented without gas
Mannitol	Fermented without gas
Mannose	Fermented without gas
Sucrose	Fermented without gas
Gelatin	Not hydrolyzed
Litmus milk	No change
Urease	Not produced
Methyl red	Negative
Hydrogen sulfide	Produced
Cytochrome oxidase	Positive
Nitrates	Reduced
Blood agar	No hemolysis
MacConkey agar	No growth
Motility	Nonmotile
Catalase	Positive

The colonial morphology of the 30 cultures isolated from humans was typical of colonies seen in cultures from birds and mammals. However, the watery mucoid colony that is often seen in cultures from the respiratory tracts of rabbits, cattle, swine, sheep, and humans has not been seen in cultures from avian species. Elberg and Ho (4) were the first to describe *P. multocida* colonies that were examined with a stereomicroscope illuminated with obliquely transmitted light. This method, which was first used for studying *Brucellae* colonies, has been

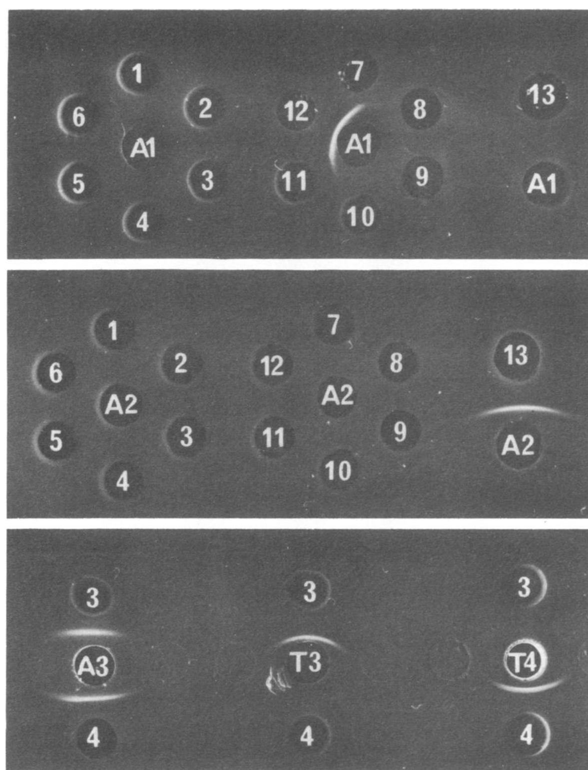

FIG. 4. *Gel precipitation patterns of P. multocida serotype 12 culture (A1), serotype 13 culture (A2), and culture (A3) that reacted with equal intensity with type 3 and 4 antisera. T3, Type 3 culture; T4, type 4 culture.*

TABLE 3. *Characteristics of Pasteurella multocida from many species of birds and mammals other than humans*

Test	No. positive/ no. tested	% Positive
Arabinose	57/1,086	5.2
Dulcitol	71/1,088	6.5
Fructose	1,099/1,099	100.0
Galactose	1,086/1,086	100.0
Glucose	1,088/1,088	100.0
Glycerol	906/1,086	83.4
Inositol	0/1,075	0.0
Inulin	0/1,083	0.0
Lactose	28/1,091	2.6
Maltose	21/1,088	1.9
Raffinose	58/1,075	5.4
Rhamnose	0/1,076	0.0
Salicin	0/1,076	0.0
Sorbitol	994/1,083	91.8
Sucrose	1,086/1,086	100.0
Trehalose	117/1,084	11.0
Xylose	820/1,087	75.4
Urease	0/1,086	0.0
Hydrogen sulfide	1,016/1,071	94.9
Indol	1,086/1,091	99.5
MacConkey agar	1/1,088	00.1

used at this laboratory for over 30 years and found to be extremely useful, particularly for selecting bacterial colonies from primary cultures from livestock and poultry.

Carter, using the indirect hemagglutination test with capsular antigens, found two serotypes (A and D) among 71 cultures of human origin; 23 cultures were nontypable, presumably because they lacked capsular antigen (2). In our studies in which we used heat-stable O antigens as serological indicators (8, 19), six serotypes were found, two of which were new. To date 16 serotypes have been found.

LITERATURE CITED

1. Bruner, D. W., and J. H. Gillespie. 1973. Hagan's infectious diseases of domestic animals, 6th ed., p. 176-184. Cornell University Press, Ithaca, N.Y.
2. Carter, G. R. 1962. Animal serotypes of *Pasteurella multocida* from human infections. Can. J. Public Health **53**:158-161.
3. DeKruif, P. H. 1921. Dissociation of microbic species. I. Coexistence of individuals of different degrees of virulence in cultures of the bacillus of rabbit septicemia. J. Exp. Med. **33**:773-789.
4. Elberg, S. S., and C.-L. Ho. 1950. Studies on dissociation in *Pasteurella multocida*. J. Comp. Pathol. Ther. **60**:41-50.

5. Gadebusch, H. H., and S. Gabriel. 1956. Modified stable Kovac's reagent for the detection of indol. Am. J. Clin. Pathol. **26**:1373–1375.
6. Gump, D. W., and R. A. Holden. 1972. Endocarditis caused by a new species of *Pasteurella*. Ann. Intern. Med. **76**:275–278.
7. Heddleston, K. L. 1972. Fowl cholera, p. 219–241. *In* M. S. Hofstad et al. (ed.), Diseases of poultry, 6th ed. Iowa State University Press, Ames.
8. Heddleston, K. L., J. E. Gallagher, and P. A. Rebers. 1972. Fowl cholera: gel diffusion precipitin test for serotyping *Pasteurella multocida* from avian species. Avian Dis. **16**:925–936.
9. Heddleston, K. L., T. Goodson, L. Leibovitz, and C. L. Angstrom. 1972. Serological and biochemical characteristics of *Pasteurella multocida* from free-flying birds and poultry. Avian Dis. **16**:729–734.
10. Heddleston, K. L., and R. C. Reisinger. 1959. Studies on pasteurellosis. III. Control of experimental fowl cholera in chickens and turkeys with an emulsified killed vaccine. Avian Dis. **3**:397–404.
11. Heddleston, K. L., K. R. Rhoades, and P. A. Rebers. 1967. Experimental pasteurellosis: comparative studies on *Pasteurella multocida* from Asia, Africa, and North America. Am. J. Vet. Res. **28**:1003–1012.
12. Henderson, A. 1963. *Pasteurella multocida* infection in man: a review of the literature. Antonie van Leeuwenhoek. J. Microbiol. Serol. **29**:359–367.
13. Holmes, M. A., and G. Brandon. 1965. *Pasteurella multocida* infections in 16 persons in Oregon. Public Health Rep. **80**:1107–1112.
14. Hubbert, W. T., and M. R. Rosen. 1970. *Pasteurella multocida* infection due to animal bite. J. Public Health **60**:1103–1108.
15. Hubbert, W. T., and M. R. Rosen. 1970. *Pasteurella multocida* infection in man unrelated to animal bite. J. Public Health **60**:1109–1117.
16. Hutyra, F., J. Marek, and R. Manninger. 1938. Pathology and therapeutics of the diseases of domestic animals, 4th English ed., p. 97. Alexander Eger Press, Chicago.
17. Moore, V. 1891. The presence of septic bacteria, probably identical with those of swine plaque, in the upper air passages of domestic animals other than swine, p. 151–162. USDA, Bureau of Animal Industry Special Report on the cause and prevention of swine plague: appendix. U.S. Government Printing Office, Washington, D.C.
18. Pasteur, M. 1879. Academic De Medecine. Bulletin **8**(2nd series):169–182.
19. Rebers, P. A., and K. L. Heddleston. 1974. Immunologic comparison of Westphal-type lipopolysaccharides and free endotoxins from an encapsulated and nonencapsulated avian strain of *P. multocida*. Am. J. Vet. Res. **35**:555–560.
20. Reisinger, R. C., K. L. Heddleston, and C. A. Manthei. 1959. A myxovirus (SF-4) associated with shipping fever of cattle. Am. J. Vet. Res. **135**:147–152.
21. Rogers, R. J., and J. Elder. 1967. Purulent leptomeningitis in a dog associated with an aerogenic *Pasteurella multocida*. Aust. Vet. J. **43**:81–82.
22. Rosen, M. N. 1970. Pasteurellosis, p. 316–223. *In* J. W. Davis, L. H. Karstad, and O. Trainer (ed.), Infectious diseases of wild mammals. Iowa State University Press, Ames.
23. Rosen, M. N. 1971. Avian cholera, p. 59–74. *In* J. W. Davis, L. H. Karstad, D. O. Trainer, and R. C. Anderson (ed.), Infectious and parasitic diseases of wild birds. Iowa State University Press, Ames.
24. Smith, J. E. 1955. Studies on *Pasteurella septica*. 1. The occurrence in the nose and tonsils of dogs. J. Comp. Pathol. Ther. **65**:239–245.
25. Smith, J. E. 1959. Studies on *Pasteurella septica*. III. Strains from human beings. J. Comp. Pathol. Ther. **69**:231–235.
26. Talbot, J. M., and P. H. A. Sneath. 1960. A taxonomic study of *Pasteurella septica*, especially of strains isolated from human sources. J. Gen. Microbiol. **22**:303–311.
27. Toussaint, M. 1879. Recl. Med. Vet. **56**:946.
28. Webster, L. T., and C. G. Burn. 1926. Biology of *Bacterium lepisepticum*. III. Physical, cultural, and growth characteristics of diffuse and mucoid types and their variants. J. Exp. Med. **44**:343–356.
29. Wessman, G. E., and G. Hilker. 1968. Characterization of *Pasteurella hemolytica* isolated from the respiratory tract of cattle. Can. J. Comp. Med. **32**:498–504.