A COMPARISON OF AEROGENIC VARIANTS OF PASTEURELLA WITH P. MULTOCIDA AND P. PNEUMOTROPICA

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ABSTRACT

A COMPARISON OF AEROGENIC VARIANTS OF *PASTEURELLA* WITH *P. MULTOCIDA* AND *P. PNEUMOTROPICA*

By

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An attempt was made to group and compare 37 strains of *Pasteurella* of various species and origin by their biochemical properties and other characteristics, including dye bacteriostasis. Of special interest in this study were the strains of *Pasteurella* which produced gas during carbohydrate fermentation.

The data which were collected by these tests were analyzed by calculating the percent similarity and arranging the strains by cluster analysis. A similarity matrix was constructed to examine the relationship between strains. All the strains of *P. multocida* of both animal and human origin formed one group. Seven of the aerogenic strains were found to resemble *P. pneumotropica* most closely, the other aerogenic strain was most like *P. multocida*.

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INTRODUCTION

Pasteurella multocida has long been recognized as a veterinary pathogen of considerable economic importance (12). It is now realized that this species is a human pathogen as well. *Pasteurella multocida* may cause local infections at the site of animal bites and may be involved in systemic infections, most often involving the respiratory and central nervous systems (24,25).

The *Pasteurella* genus is a very complex one taxonomically. There have been many studies to examine the interrelationships of these species and to improve the classification (39,40,50,54). These studies have led to a simplification of the genus and the reclassification of some species to another genus (50).

In this study, the strains which were examined were *P. multocida* of animal and human origin, the closely related genus of *P. pneumotropica*, and the aerogenic variants of *Pasteurella* which were tentatively identified as *P.* "gas" (*multocida*?). Test strains of *P. ureae* and *P. hemolytica* were also included for comparative purposes. These strains were compared by means of biochemical and other tests. The biochemical tests were those recommended for Gram negative rods (28,56). In addition, dye sensitivity tests were carried out on these strains in order to see if a repeatable specific pattern of susceptibility for each species or variety could be established. This had been tried previously without success (46).

The data which were collected through these methods were analyzed by the calculation of the percent similarity and were arranged into groups by cluster analysis (2,52). The groups were depicted diagrammatically by a similarity matrix which provided a means of comparing each strain individually with the other strains.

REVIEW OF THE LITERATURE

Pasteurella, which was created by Trevisan (54) to include the agent of fowl cholera and hemorrhagic septicemia bacilli, is a genus in the family, *Brucellaceae*. This genus is defined as small Gram-negative rods which generally occur in pairs or short chains and which are characterized by bipolar staining, pleomorphism, absence of gelatin liquification, and the fermentation of carbohydrates with slight acid production (4). In the 1957 edition of *Bergey's Manual* (4), there are the following 9 species listed for this genus: *P. multocida*, *P. septicaemiae*, *P. hemolytica*, *P. anatipestifer*, *P. pestis*, *P. pfaffi*, *P. pseudotuberculosis*, *P. tularensis*, and *P. novicida*.

These 9 species form a very heterogeneous group of organisms. Taxonomic studies have been attempted to understand the interrelationships of these species and the others which have been added to the genus and to improve the classification (39,40,50,54).

Since the 1957 publication of *Bergey's Manual*, *P. tularensis* (40) and *P. novocida* (39) were reclassified as belonging to the genus, *Francisella*. *P. pfaffi*, *P. septicaemiae*, and *P. anatipestifer* were considered questionable members of this genus. *P. pfaffi* is not readily distinguishable from *P. pseudotuberculosis*, and *P. septicaemiae* and *P. anatipestifer* are gelatin liquifiers which contradicts the definition of the genus (50).

However, even with these simplifications, the remaining species still provide some difficulty in classification. Work by Talbot and Sneath (54) showed that *P. multocida* is only distantly related to

P. pestis and *P. pseudotuberculosis*, but the latter species are closely related to each other. Smith and Thal (50), in 1965, confirmed these results when they employed the Adansonian method to classify 25 strains of *Pasteurella* of various species. They found that the strains fell into 2 broad groups on the basis of the oxidase reaction. Group I which is oxidase positive included: *P. multocida*, *P. pneumotropica*, *P. hemolytica*, and *P. hemolytica var. ureae*. Group II which is oxidase negative consisted of: *P. pseudotuberculosis*, *P. pestis* and *P. "X"*. Group II has been reclassified as belonging to the genus, *Yersinia* (50).

In Group I, the 2 most dissimilar species are *P. multocida* and *P. hemolytica*. They were recognized early in experimental studies as 2 distinct groups within the *Pasteurella* genus. The atypical hemolytic, non-indole forming group was named *P. hemolytica* (37). Later, the non-hemolytic, indole positive organism was called *P. multocida* (46).

Pasteurella multocida

Pasteurella multocida is a heterogeneous species. Using computer taxonomic technique, strains of this species have shown an 85% similarity with each other (50). Therefore, there have been repeated attempts to subdivide the species.

Nomenclature

The name, *P. multocida*, is a recent one having been proposed by Rosenbusch and Merchant in 1939 (46). It has its origin in the first name, *Bacterium bipolare-multocidum*, given to hemorrhagic septicemia organisms isolated from cattle, swine, deer, horses and goats by Kitt in 1885 (46). The large number of species names which appeared in the early literature is a direct result of the wide variety of animals infected and clinical diseases produced.

The diversity of infection prompted an effort to subdivide these organisms according to a zoological classification (30). This system proved disadvantageous because organisms which caused a single type of disease were given a wide variety of names. The zoological classification was later abandoned as a result of many cross-pathogenicity and cross-immunity studies which proved that this was not a valid means of subgrouping these organisms (42). Even at the present time, the species name is not uniform. Topley and Wilson introduced the name *P. septica* in the *Principles of Bacteriology and Immunology*, 1st edition, 1939, p. 48. This name appears widely throughout the literature of Great Britain and other Commonwealth nations.

Cellular Morphology

Pasteurella multocida is a Gram-negative organism which varies from a long rod to a coccobacillus (12). Smith (48) recorded small differences in the cellular morphology of cultures from different animal sources. He found the bovine strains to be pleomorphic measuring 0.5 by 1.2 μ, while porcine strains were non-pleomorphic about 0.8 by 1.0 μ. Dog strains varied from small coccobacilli to long S-shaped filaments. These differences have not been found to be adequate or precise distinctions. Cultures from all animal sources, especially those maintained on laboratory stock culture media for a long period of time, tend to form long filamentous forms (12).

Colonial Morphology

Several colonial variants of *P. multocida* have been demonstrated. The terminology which has been used to describe these variants often differs, but the 3 main variants have been described as: iridescent (smooth), blue (rough), and mucoid (14,16).

To distinguish these variants a common method is to observe the colonies through obliquely transmitted light. The smooth colonies will appear iridescent with a greenish tint, while mucoid colonies are reddish iridescent, and the rough colonies appear blue (16).

The 3 colonial variants also differ in physical appearance on a blood plate. The mucoid colonies are the largest with irregular margins, while the smooth and rough colonies are smaller with entire edges (12). The iridescent and mucoid variants are most often obtained from animals with acute diseases, and the blue variants are isolated frequently from animals with chronic infections (7,14).

Attempts (18,13) have been made to correlate colonial variation with other properties of the organism. It has been experimentally determined that there is a relationship between the colonial morphology and virulence. Iridescent colonies are the most virulent of the colonial variants for the mouse, and rough colonies vary in their virulence for mice (13).

Although colonial variation does affect virulence and serological behavior, it seems to have no other significant effect. Biochemical characteristics including carbohydrate fermentation and indole production are not affected by colonial variation (18).

Biochemical Reactions

In a biochemical description of a species, the fermentation pattern of carbohydrates is significant. A "typical" pattern for *P. multocida* (46,48,54), using 1% peptone water and Andrade's indicator, is acid from glucose and sucrose and no acid production, with rare exceptions, from lactose, arabinose, dulcitol and inositol. Approximately 50% of the strains tested produced acid in xylose, mannitol and sorbitol, while maltose is only rarely fermented. *Pasteurella multocida* does not produce

gas routinely in carbohydrate fermentation; however, there are reports in the literature of a few aerogenic strains (45,54).

Talbot and Sneath (48) described a strain of *P. multocida*, isolated from a human dog bite infection, which produced gas in small quantities from sucrose, maltose, mannose and trehalose. There was also a report of an aerogenic strain from a purulent leptomeningitis in a dog (45). In all other respects these strains were typical of *P. multocida*. In addition, Dr. Weaver (56) in his classification chart for Gram-negative organisms included a group of *Pasteurella* which produced small quantities of gas from glucose. These strains did possess other deviant reactions and were tenatively identified as *P.* "gas" (*multocida*?). This phenomenon of gas production has not been studied by taxonomists in any further detail.

The fermentation pattern of *P. multocida* is notable in its variability. Some of the variation in certain carbohydrates may result from the comparison of results obtained by the use of different growth media and indicator systems. It has been found in a closely related species, *P. pneumotropica*, that with certain sugars which are slowly fermented varying the indicator will alter the outcome of the test (20). Certain variation from the norm has been observed to predominate in one host species over another. Smith (48) found that strains from cattle and pigs generally ferment xylose, sorbitol and mannitol, while dog strains usually ferment maltose, trehalose, and dextrin.

In considering the biochemical reactions of a bacterial species the fermentation pattern is studied because it is thought of as a stable property. In *P. multocida*, there is some evidence that it is a dynamic property. Rosenbusch and Merchant (46) observed 4 strains which showed changes in their fermentation of xylose, arabinose and dulcitol over a

2-year period. In the more recent work of Higgins (22), he noticed a change in the fermentation pattern of a strain which was causing an outbreak of fowl cholera in a chicken flock. This has led to some speculation of an interrelationship of virulence, antigenic characteristics, and carbohydrate fermentation (22).

In addition to carbohydrate fermentation, certain other biochemical reactions may be used to characterize the species (12,28,46,50,54). Catalase, oxidase, and indole are produced and nitrates are reduced to nitrites. The methyl red and Voges-Proskauer tests are negative. Hydrogen sulfide is produced in small amounts which is detectable by suspending a lead acetate strip over a triple sugar iron agar slant. Urease production is generally negative, but it can be positive in a few rare cases. Citrate and malonate are not utilized, and gelatin is not liquefied. Of the decarboxylase reactions, only ornithine decarboxylase is positive.

Serological Studies of *Pasteurella multocida*

Many workers have attempted to classify *P. multocida* through serological techniques. Some of the early workers in this field included Cornelius, Ochi, Yusef, and Khalifa (12). One of the most important of the early studies was the work of Rosenbusch and Merchant (46). They divided strains of *P. multocida* into 3 groups according to its fermentation of xylose, arabinose, and dulcitol and agglutination tests. In general, the early studies underestimated the complexity of the species and used many different standards to evaluate and classify the organisms (12).

The next attempt at a serological classification of the species was the work of Little and Lyon (31). They divided 30 strains of

non-hemolytic *Pasteurella* by slide agglutination test into 3 types. However, it is now appreciated that this typing procedure was inadequate (12). Another method to subgroup *Pasteurella* was with the serum protection test in mice. In 1947, Roberts (44) used this method to separate 37 strains into 4 types which were numbered with Roman numerals I through IV. This classification was found to loosely parallel some host and biochemical differences. Types I and III did not ferment arabinose but was xylose positive. Type II fermented arabinose but generally did not ferment xylose. Type IV fermented arabinose and dulcitol, but there was an uncertain reaction pattern with xylose.

At the present time a serological classification of *P. multocida* has been established which uses the combined capsular and somatic antigenic make-up to categorize the species. The capsule of *P. multocida* consists of protein and carbohydrate components (1,43). Both the protein-associated capsular substance (1) and the capsular polysaccharides (6, 29,42) have been demonstrated to have serological activity, but the polysaccharide component alone is not immunogenic. Through an indirect hemagglutination test (8) in which the capsular extracts were adsorbed to the erythrocytes, 5 serotypes were named. The serotypes were given the letters A through E. It was later suggested that the group C be dropped because it did not adequately represent a capsular type (10).

In addition to the capsular system, Namioka and Murata (36) further classified strains on the basis of somatic or O antigens. This and the work of Namioka and Bruner (35) divided *P. multocida* into 11 O groups. Each O group is designated by an arabic number. If the O groups and the capsular types are correlated, 14 combined serotypes result (12). Although this dual serological classification promises to add to the present knowledge of *P. multocida*, it is acknowledged that the multiple

cross reactions, especially in determining the O serotype, can cause difficulty (35).

Animal Infection

Pasteurella multocida is known as an animal pathogen of considerable economic importance. The pathogenicity of the organism is variable. It is involved in infections which vary from the highly severe virulent forms to non-clinical commensalism in which organisms are found in the nasopharynx (12). The organism also acts as a secondary invader in a number of viral and mycoplasmal infections (12).

The mechanism of infection of this organism is not completely understood. Stresses of various kinds are important, especially in the periodic outbreaks of shipping fever of pasteurellosis and fowl cholera (11). It seems most probable that the process of endotoxin poisoning is responsible for the pathologic effects in acute septicemias and death (12). The organism is transmissible by contact, contaminated food and water and in some cases by droplet infection (33).

The principal acute infections in animals are fowl cholera, hemorrhagic septicemia in cattle and water buffalos, enzootic pneumonia in swine, snuffles in rabbits, and periodic infections of a wide range of animals (12). Severe acute septicemic infection results in such clinical signs as blood vascular congestion, submucous and subserous hemorrhage and enteritis (11). The chronic type of infection in a localized area is characterized by abscessation and by anemia and diarrhea leading to a general debilitation (11).

Human Infection

It is becoming apparent that *P. multocida* must be considered a human as well as an animal pathogen. The first infection of man with

P. multocida was reported by Brugnatelli in 1913 (15). It was isolated from a puerperal pyrexia. In 1947, Schipper summarized the literature of *Pasteurella* infections from 1930-1947 reporting approximately 40 cases (15). Meyer in 1955 updated this review counting 95 cases to that year (15). An additional 12 cases were counted by Swartz and Kunz (58) from 1955-1959.

There has been a considerable increase in reporting of isolations from humans in the last few years due to increased familiarity with the species (9). The relatively small number of cases in earlier literature is probably the result of errors in diagnosis. This is dramatically shown by an 8-month study at the University of Colorado, in which 10 cases were reported in this short time period (17).

The largest number and the most thoroughly studied infections in humans are in the local infections which result from bites of dogs, cats, and other mammals (15). It has been proven that *P. multocida* is a common commensal of the respiratory tract of domestic animals (48). These organisms may be injected into the wound at the time of the bite.

An epidemiological study (24) has been made of local *P. multocida* infections to see if any trends in infection do exist. According to this study, these infections are most frequently located on the upper extremities, and 73% of the people infected were 40 years of age or older. The infection rate resulting from cat bites was about 10 times greater than dog bites. The lower frequency of dog bite infections may be accounted for by certain differences between dog and cat strains. Cultures isolated from the throats of dogs were usually rough (49).

The appearance of clinical symptoms in infections occurs very quickly. In about 24 hours, the patient feels severe pain and swelling and has a gray odorous discharge at the site (3). If the patient does not seek

medical treatment, recovery may be slow and there may be underlying necrosis of the bone (49). The underlying bone involvement is a more frequent problem if the organism has been injected into a poorly vascularized region. The result may be a chronic osteomyelitis, cellulitis, and adenitis with abscess formation (3,22).

For these local infections, the treatment is usually antibiotic therapy (32,58). *Pasteurella multocida* is a Gram-negative organism which is highly sensitive to penicillin. In some cases surgery may be needed (19). This is especially true if the bone becomes extensively involved.

In analyzing the biochemical reactions of cultures isolated from human infections, it has been reported by Smith (49) that the cultures isolated from dog bite wounds resemble strains isolated from the throat of healthy dogs. The same relationship appeared from strains isolated from cat infections. However, there is some contradictory data in the literature (17,32) which show the isolation of mannitol positive strains from local human infections. This is not characteristic of dog strains isolated from healthy dogs.

A second type of human infection with *Pasteurella* is the systemic infection which is unrelated to animal bites. The most frequently diagnosed conditions in these infections are meningitis, empyema and bronchiectasis (25).

The largest group of internal infections are the infections of the respiratory system (47). The classic patient who develops a pulmonary infection with *P. multocida* is an individual who has had many years of contact with animals and has some underlying chronic respiratory problems (47). Systemic infections may also involve systems other than the respiratory system. The non-respiratory systemic infections often involve the

appendix and the central nervous system (25). The 2 most common clinical conditions are meningitis and chronic otitis media (25).

A mechanism of pathogenicity has been suggested for the non-respiratory infections. The organism may reside as a commensal in a healthy respiratory tree until some trauma to the host system causes it to invade the other host tissue (15). This is supported by the fact that there are reports of humans carrying this organism symptomless in their respiratory tract (49). A site such as a carcinoma, appendiceal abscess or any perforated abscess may also provide *P. multocida* with an opportunity to act as a secondary invader (15).

In the systemic infections with *P. multocida*, septicemia may or may not occur, but if it does occur chances for recovery are poor (15). The general treatment for these infections as with the local infections is antibiotic therapy (15). There has been only one report of a penicillin resistant strain (47).

In *P. multocida* infection, an animal source is always presumed. However, there have been reported cases where such a source could not be found (25). It has been postulated that in addition to animal to human infection, there might also be a human to human route of infection (25).

Isolation of *P. multocida* from Human Clinical Specimens

In the human diagnostic laboratory, *P. multocida* can be confused with certain other Gram-negative rods. In direct smears from certain specimens, pleomorphic forms can be found which resemble *Hemophilus* (3). *Pasteurella multocida* can be differentiated from *Hemophilus* by the fact that *P. multocida* grows in the absence of X and V factors and is sensitive to penicillin (3).

Meningeal smears may show Gram-negative diplococci which can be mistaken for *Neisseria* and *Mima* (15). The latter are, like *P. multocida*, oxidase positive. *Pasteurella multocida* can be distinguished by the fact that it produces indole and reduces nitrate while *Neisseria* and *Mima* do not (3).

Pasteurella multocida is sometimes overlooked in isolations from animal bites or other places from which enteric bacteria are expected. One reason that *P. multocida* is frequently overlooked is because it reduces nitrate and may resemble enteric bacilli in broth. However, it is distinguished as a separate species, by the fact that it does not grow in the presence of bile salts, is oxidase positive and is sensitive to penicillin (2).

The main characteristics which should indicate that *Pasteurella* might be involved in the infection is: the source of the swab, e.g., cat or dog bite, penicillin sensitivity, morphology and Gram-negativity (51).

Pasteurella pneumotropica

Pasteurella pneumotropica is the species of *Pasteurella* which is the most closely related in its biochemical characteristics to *P. multocida*. It was first isolated and described by Jawetz in 1950 (27).

Biochemical Reactions

Pasteurella pneumotropica appears as a convex, round, entire, semi-translucent colony about 1 mm. in diameter with a smooth surface which varies in color from white to grayish yellow (5,27,57). There is no hemolysis on horse, sheep or rabbit blood, but there is occasionally greening under the colonies (5,27,57). Similar to *P. multocida*,

P. pneumotropica is indole, nitrate, catalase and oxidase positive, and MacConkey, methyl red, and Voges-Proskauer negative (20,23,57). *Pasteurella pneumotropica* differs from *P. multocida* chiefly in that it is urease positive and ornithine decarboxylase negative (57). In addition, *P. pneumotropica* is more susceptible to penicillin than *P. multocida.*

The carbohydrate fermentation pattern is also slightly different for the 2 species. Using the same test system which was used to test for the fermentation pattern of *P. multocida*, Andrade's indicator with 1% peptone water, *P. pneumotropica* produces acid but no gas from glucose, xylose, lactose, sucrose, maltose and arabinose (21). It does not ferment mannitol, sorbitol, dulcitol, and inositol (21).

Serological Studies

In serological studies of *P. pneumotropica*, normal mice who carried this organism in the latent form in their lungs had no agglutination or complement fixation titer (23). After experimental inoculations, all the mice had complement fixation titers of 1:10 but no agglutination titers (23). Cross-absorption tests indicated that there were some antigenic differences between strains isolated from different animal colonies which suggested an antigenic mosaic similar to *P. multocida* (27). In further studies, *P. pneumotropica* cells were not agglutinated by antisera of *P. pestis*, *P. pseudotuberculosis*, *P. multocida*, *B. bronchiseptica*, *H. influenza*, type B, and *H. pertussis* (27). However, interaction of *P. multocida* and *P. pneumotropica* cannot be ruled out completely because antisera for all the serotypes of *P. multocida* have not been tried.

Pathogenicity

In its initial isolation *P. pneumotropica* was characterized as a latent organism which is an inhabitant of the lungs of mice (27). After 14 serial passages in mice, the infectivity was still very low. This species caused lesions only in the lung and only when injected intranasally. The only other injection route which caused any disease process was intracerebral injections which caused brain abscesses (27). The virulence of the organism obtained by serial passage was unstable (27). Repeated transfer on artificial media soon resulted in a partial loss of pathogenicity (27).

Jawetz speculated that *P. multocida* and *P. pneumotropica* might share a common ancestor and represent 2 ends of a bacterial spectrum. *Pasteurella multocida* at one end of the spectrum is an invasive organism of potentially high virulence and wide host specificity, and *P. pneumotropica* is at the other end with tissue specificity and low virulence. He suggested that the different biochemical features might be the result of the gradual adaptive changes between the host and the parasite (27).

Additional experimentation has shown that *P. pneumotropica* has a wider spectrum of pathogenicity and greater virulence than first thought. In the mouse, it was found in the latent form in the brain, uterus, liver as well as the lung (23). *Pasteurella pneumotropica* has also been isolated from a number of other animals including rats, kangaroo rats and hamsters (5).

Pasteurella pneumotropica can also be found associated with natural disease processes. It was found in the lungs of rats and mice with pneumonia (5) and from the eyes of mice with conjunctivitis (5). In dogs, it has been associated with otitis, peritonitis, renal abscess, pneumonia, kidney abscess and septicemia (5). There have also been records

of human respiratory tract isolations (20) and isolations from local inflammation at the site of a dog bite (57) and cat bite (58).

METHODS AND MATERIALS

Cultures

Thirty-seven strains of *Pasteurella* were studied. The strains which were isolated from cases of human pasteurellosis were obtained from 2 sources. Thirteen strains were provided by the Michigan Department of Public Health, Bureau of Laboratories, Lansing, Mich. Six additional cultures of human pasteurellosis isolates were supplied by Dr. Robert E. Weaver of the Center for Disease Control, Atlanta, Georgia.

The 12 strains of *Pasteurella multocida* of animal origin studied were part of the stock culture collection of Dr. G. R. Carter, Michigan State University. Four cultures of *Pasteurella* of species other than *Pasteurella multocida* were also obtained from this collection. Two strains of *Pasteurella pneumotropica* were received from the laboratory of Dr. Frederickson, Copenhagen, S. Denmark.

All the stock cultures were maintained on Difco stock culture medium. The cultures were transferred to fresh stock culture medium at 3-month intervals.

Colonial Iridescence

Each strain was grown on BBL trypticase soy agar for 24 hours. If the colonies were iridescent, it could be seen clearly when the plates were examined in a darkened room with a strong oblique light falling on the plate from behind.

Biochemical Reactions

Each strain was streaked for isolated colonies on blood agar plates which consisted of BBL trypticase soy agar base and approximately 7% defibrinated bovine blood. Colonial morphology at 24 hours was noted. Growth from these plates was Gram-stained and was used to inoculate the other biochemical media. The biochemical tests, in general, were incubated at 37° and were read at 24 hours, 48 hours, and 7 days.

Carbohydrate utilization was studied with 3 types of media. Tests were carried out in semi-solid phenol red broth containing Difco phenol red base, 0.15% agar, and 1% of the particular carbohydarte. Oxidation-fermentation test was performed using the method of Hugh and Leifson (26) with various carbohydartes as substrates. The growth on 10% glucose and lactose slants was also recorded.

Difco Simmon citrate slants were used to test for citrate utilization, while Christensen urea slants were used for urease production. H_2S production was detected by suspending lead acetate strips over triple sugar iron agar slants. Esculin hydrolysis was studied by using the modified esculin agar recommended by Sneath (51). Decarboxylase tests for lysine and ornithine and the arginine hydrolase test were performed using Moeller decarboxylase broth (34).

Oxidase production was detected with a few drops of a 0.5% solution of tetramethyl-p-phenylene-diamine dihydrochloride on 24 hours growth on a blood plate. For the catalase test, a few drops of 3% hydrogen peroxide on 24 hours growth on TGY slants were used.

Nitrate reduction, indole production and the MR/VP tests were performed after 48 hours' incubation. Indole production was examined using Kovac's method. Nitrate reduction was tested by adding 3 drops of 0.8% sulfanilic acid and 5 N acetic acid mixture and 3 drops of a 0.5% alphanaphtholamine and 5 N acetic acid mixture.

All the media and reagents used in the tests referred to above and the other biochemical reactions studied were obtained from the Difco Company or Baltimore Biological Laboratories.

Dye Sensitivity

Cultures. All the strains for dye sensitivity testing were grown in Difco beef heart infusion broth at 37° C. for 18 to 24 hours.

Preparation of Dye Discs. Stock solutions of pyronin (1:500), methyl violet (1:100), basic fuschin (1:100), and thionin (1:200) were made up in the specified concentrations (w/v) in 50% ethyl alcohol. Corrections were made for the dye impurities. The stock solutions were stored in the dark for no longer than 2 months at 4° C.

Taking care to maintain asepsis the dye discs were prepared by completely saturating Difco Bacto concentration discs sterile blanks (1/2" in diameter) with dye solution. The saturated discs were allowed to dry in sterile containers in the 37° incubator.

Blood plates were used for the disc sensitivity testing. Sterile swabs were saturated with the broth culture. The plates were inoculated by swabbing the blood plate in 3 directions. This procedure was shown in control plates to yield confluent growth. The dye discs were then placed on the inoculated plates with sterile forceps. The plates were read as millimeters of inhibition from the edge of the dye disc. A zone of inhibition of 4 mm. or more was recorded as a positive.

Numerical Analysis

The reaction of the strains were scored using method III of Beers *et al.* (56). In this procedure, the features to be examined are outlined, and each strain is scored as + if it possesses the characteristic, and

- if it does not possess it. The designation, 0, is used when the characteristic does not apply to the individual strain. Table 1 contains a list of characteristics tabulated in this study.

Percent similarity coefficients (S) were calculated for each possible pair of strains by counting n which is the number of times + signs coincide and n' which is the number of times + and - signs coincided, and substituting in the formula:

$$S = \frac{100\ n}{n + n'},$$

0 signs were ignored.

The strains were sorted manually according to the methods of cluster analysis described by Sneath (52). The groupings which resulted were depicted diagrammatically by a similarity matrix.

Table 1. List of characteristics for numerical analysis

1.	24 hr. colonial morphology: clear-white colony, 0.5-1.0 mm. round, raised, entire, buttery, shiny.
2.	24 hr. colonial morphology: clear-white colony, pinpoint-0.5 mm., round, raised, entire, transparent.
3.	Gram stain: Gram negative coccobacillus.
4.	Gram stain: Gram negative rod.
5.	Gram stain: Gram negative rod with pleomorphism.
6.	Fermentation of 1% glucose.
7.	Fermentation of 1% xylose.
8.	Fermentation of 1% lactose.
9.	Fermentation of 1% mannitol.
10.	Fermentation of 1% sucrose.
11.	Fermentation of 1% maltose.
12.	Fermentation of 1% sorbitol.
13.	Fermentation of 1% arabinose.
14.	Fermentation of 1% inositol.
15.	Fermentation of 1% dulcitol.
16.	Gas production from carbohydrates.
17.	Fermentation of 10% glucose.
18.	Fermentation of 10% lactose.
19.	Catalase production.
20.	Oxidase production.
21.	Growth in nutrient broth.
22.	Growth on MacConkey's agar.
23.	Growth on SS agar.
24.	Growth on Cetrimide agar.
25.	Utilization of citrate.
26.	Production of urease.
27.	Nitrate production.
28.	Indole production.
29.	Acid production in 48 hrs. on TSI slant.
30.	Acid production in 48 hrs. on TSI butt.
31.	Darkening of lead acetate in 48 hrs.
32.	Methyl red test.
33.	Voges-Proskauer test.
34.	Gelatin liquification.
35.	Motility.
36.	Esculin hydrolysis.
37.	Growth on TGY slant at 25° C.
38.	Growth on TGY slant at 37° C.
39.	Growth on TGY slant at 42° C.
40.	Inhibition of growth by pyronin (1:500).
41.	Inhibition of growth by methyl violet (1:100).
42.	Inhibition of growth by basic fuschin (1:100).
43.	Inhibition of growth by thionin (1:200).

RESULTS

Table 2 presents descriptions of strains used in this work. From this initial information, the cultures could be subdivided into the following 5 classes: (1) *P. multocida* of human origin; (2) *P. multocida* of animal origin; (3) *P. pneumotropica*; (4) *Pasteurella* of other species; and (5) *P.* "gas" (*multocida*?).

Table 3 is a tabulation of the results of biochemical and other tests according to these 5 classes. The strains of *P. hemolytica* and *P. ureae* can be distinguished by their lack of production of indole and their fermentation reactions. *Pasteurella pneumotropica* can be separated from *P. multocida* by its production of urease and its carbohydrate fermentation pattern. *Pasteurella pneumotropica*, unlike *P. multocida*, consistently fails to ferment mannitol and sorbitol, but it does ferment maltose. The biochemical reactions of *P.* "gas" (*multocida*?) appear more closely related to *P. pneumotropica* than *P. multocida*. There is no significant difference among strains isolated from animal and human infections of *P. multocida*.

In Table 3, dye sensitivity testing with discs does give significantly consistent and different patterns for *P. multocida*, *P. pneumotropica*, and *P.* "gas" (*multocida*?). This technique is one that has not been used to distinguish *Pasteurella* species.

The above 5 classes proved to be an inadequate way to represent the biochemical data obtained for these organisms. For example, in addition to the 6 aerogenic strains provided by Dr. Weaver, 2 other isolates,

Table 2. Description of *Pasteurella* strains

No.	Strain no.	Species origin	Source	Disease and/or specimen source	Colonial variant
1	CD-90A	Human	Mich. Dept. of Public Health	Human septicemia (fatal)	Iridescent and blue
2	CD-623	Human	Mich. Dept. of Public Health	Brain abscess	Blue
3	CE-154	Human	Mich. Dept. of Public Health	Oida septicemia	Blue
4	CC-1193	Human	Mich. Dept. of Public Health	Dog bite (face)	Blue
5	CC-1213	Human	Mich. Dept. of Public Health	Dog bite (leg)	Blue and iridescent
6	CE-28L	Human	Mich. Dept. of Public Health	Dog bite (leg)	Blue
7	CC-1183	Human	Mich. Dept. of Public Health	Wound drainage	Blue
8	CD-1058	Human	Mich. Dept. of Public Health	Wound infection	Iridescent
9	CC-1043	Human	Mich. Dept. of Public Health	Cat bite	Blue and iridescent
10	CC-971	Human	Mich. Dept. of Public Health	Cat scratch	Blue and iridescent
11	OL-143	Human	MSU Vet. Micro. Lab.	Opossum bite (hand)	Blue and iridescent
12	CE-49	Human	Mich. Dept. of Public Health	Tiger bite	Blue

Table 2 (cont'd.)

No.	Strain no.	Species origin	Source	Disease and/or specimen source	Colonial variant
13	CC-1150	Human	Mich. Dept. of Public Health	Sputum	Iridescent
14	B-92	Human	Dr. Weaver	Dog bite	Blue
15	B-767	Human	Dr. Weaver	Dog bite	Blue
16	A-8708	Human	Dr. Weaver	Dog bite	Blue
17	B-704	Human	Dr. Weaver	Foot wound	Blue
18	B-972	Human	Dr. Weaver	Ear	Blue
19	B-1682	Human	Dr. Weaver	Leg wound	Blue
20	Insein B	Bovine	MSU Vet. Micro. Lab.	Hemorrhagic septicemia	Blue and iridescent
21	P-1235	Bovine	MSU Vet. Micro. Lab.	Unknown	Blue and iridescent
22	r-473	Bovine	MSU Vet. Micro. Lab.	Hemorrhagic septicemia	Blue
23	19427	Unknown	MSU Vet. Micro. Lab.	Unknown	Blue
24	932A	Bovine	MSU Vet. Micro. Lab.	Unknown	Blue
25	3397	Porcine	MSU Vet. Micro. Lab.	Unknown	Iridescent
26	M-17	Sheep	MSU Vet. Micro. Lab.	Pneumonia	Blue and iridescent
27	C-566	Canine	MSU Vet. Micro. Lab.	Unknown	Blue
28	C-55	Canine	MSU Vet. Micro. Lab.	Unknown	Blue

Table 2 (cont'd.)

No.	Strain no.	Species origin	Source	Disease and/or specimen source	Colonial variant
29	F-24	Feline	MSU Vet. Micro. Lab.	Unknown	Blue
30	P-1059	Avian	MSU Vet. Micro. Lab.	Fowl cholera	Blue and iridescent
31	M-355	Opossum	MSU Vet. Micro. Lab.	Throat swab	Blue
32	*P. hemo.* B-128-71	Bovine	MSU Vet. Micro. Lab.	Pneumonia	Iridescent
33	*P. ureae*	Human	MSU Vet. Micro. Lab.	Bronchiectasis	Iridescent
34	*P. pneumo.* M-199-70	Rat	MSU Vet. Micro. Lab.	Unknown	Blue and iridescent
35	*P. pneumo.*	Rat	MSU Vet. Micro. Lab.	Unknown	Blue
36	P-421	Unknown	Dr. Frederickson	Unknown	Blue
37	P-309	Unknown	Dr. Frederickson	Unknown	Blue

Key:

* - Positive reaction

\- - Negative reaction

A. - Acid production

Alk. - Alkaline

( ) - Delayed reaction

(No.) - Number of deviant strains

Table 3. Summary of biochemical tests

	Test	*P. multocida* (13) (human)	*P. multocida* (12) (animal)	*P. "Gas"* (*multocida*?) (6)	*P. pneumotropica* (4)	*P hemolytica* (1)	*P. ureae* (1)
	Colonial Morph. (24 hrs.)	clear round 0.5-1 mm. raised entire buttery shiny	clear-white round pinpoint-1.5 mm. raised entire buttery shiny	clear-white round 0.25 mm. raised entire buttery shiny	white round pinpoint 1 mm. raised entire buttery shiny	clear round 0.5 mm. raised entire buttery shiny	grayish white round 1 mm. raised entire buttery shiny
	Gram Stain	Gram-cocco-bacilli-pleomorphic rods	Gram-cocci-pleomorphic rods	Gram-cocco-bacilli-pleomorphic rods	Gram-cocco-bacilli-pleomorphic rods	Gram-diplococci	Gram-pleomorphic rods
	Action on Blood	-	-	-	-	-	-
	OF	F	F	F	F	F	F
	F						
S	Glucose	+	+	+	+	+	+
E	Xylose	+(3)	+(5)	-	+(2)	+	-
M	Lactose	-(1)	-	-	-	+	-
I	Mannitol	+(2)	+(2)	-	-(1)	+	+
S	Sucrose	+(1)	+	+	+	+	+
O	Maltose	-	-(2)	+	+	+	+
L	Sorbitol	+(2)	+(2)	-	-	+	-
I	Arabinose	-	-(1)	-	-(1)	+	-
D	Inositol	-	-	-	-(2)	+	-
	Dulcitol	-	-	-	-	-	-
	Glucose	A/-(1)	A/-(1)	A/+(1)	A/-	A/-	A/-
	Xylose	A(3)/-	A(4)/-	(-/-)	-/-	A/-	-/-
D	Lactose	-(1)/-	-/-	-/-	-/-	A/-	-/-
U	Mannitol	A(2)/-	A(3)/-	-/-	-(1)/-	A/-	A/-
R	Sucrose	A(1)/-	A/-(1)	A/+(3)	A/-	A/-	A/-
H	Maltose	-/-	-(1)/-(1)	A/+	A/-	A/-	A/-
A	Sorbitol	A(2)/-(1)	A(3)/-	-/-	-(2)/-	A/-	-/-
M	Arabinose	-/-	-(1)/-	-/-	-(1)/-	A/-	-/-
	Inositol	-/-	-/-	-/-	-(2)/-	A/-	-/-
	Dulcitol	-/-	-/-	-/-	-/-	-/-	-/-

Table 3 (cont'd.)

Test	*P. multocida* (13) (human)	*P. multocida* (12) (animal)	*P.* "Gas" (*multocida*?) (6)	*P. pneumotropica* (4)	*P. hemolytica* (1)	*P. ureae* (1)
10% Glucose	A	A	A	A	A	A
Lactose	Alk.(1)	Alk.	Alk.	Alk.(1)	A	Alk.
Catalase	+	+	+	+	+	+
Oxidase	+	+	+	+	+	+
Nut. Br.	+	+	+	+	+	+
MacConkey	-	-	-	-	-	-
SS	-	-	-	-	-	-
Cetrimide	-	-	-	-	-	-
Citrate	-	-	-	-	-	-
Urea	-	-(1)	+	+	-	+
NO_3	+	+	+	+	+	+
Indole	+	+(1)	+	+	-	-
Slant	24-48 hr. A(1)	24-48 hr. A	48 hr. A	24 hr. A	24 hr. A	24 hr. A
TSI	7 days A(1)	7 days A	7 days A	7 days A	7 days A	7 days A
Butt	24-48 hr. A	24-48 hr. A	48 hr. A	24 hr. A	24 hr. A	24 hr. A
	7 days A(1)	7 days A	7 days A	7 days A	7 days A	7 days A
Paper	24-48 hr. trace-+	24-48 hr. +	24-48 hr. +	24 hr. +	24 hr. +	24 hr. - 48 hr. -
H_2S	7 days +	7 days +	7 days +	7 days +	7 days +	7 d. -
Butt	7 days -	7 days -	7 days -	7 days -	7 days -	7 days -
MR/VP	-/-	-/-	-/-	-/-	-/-	-/-
Gelatin	-	-	-	-	-	-
Litmus Milk	-	-	-	-	-	-
Motility	-	-	-	-	-	-
Esculin hydrolysis	-	-	-	-	-	-
25° C.	+	+	+	+	+	+
TGY 37° C.	+	+	+	+	+	+
42° C.	-	-	-	-	-	-
Arginine	-	-	-	-	-	-
Lysine	-	-	-	-	-	-
Ornithine	+(1)	+(2)	-	+	-	-
Flo Agar	-	-	-	-	-	-
Tech Agar	-	-	-	-	-	-
Pyronin	+(2)	+(1)	-(1)	-(1)	+	+

Table 3 (cont'd.)

Test	*P. multocida* (13) (human)	*P. multocida* (12) (animal)	*P. "Gas"* (*multocida*?) (6)	*P. pneumotropica* (4)	*P. hemolytica* (1)	*P. ureae*
Methyl violet	-(3)	-(2)	+(1)	-(1)	-	+
Basic Fucshin	-(1)	-(2)	+	-	-	+
Thionin	+(2)	+(1)	-(1)	+	+	+

strain 12 and strain 27 were also gas producers. Table 4 tabulates the gas production data observed using a Durham tube. In addition to the reported production of gas from glucose (56), it was also observed from other carbohydrates which these strains fermented. Three of the strains showed a more limited ability to produce gas. A control tube of phenol red base alone was used to eliminate the possibility of a non-specific breakdown of another substance in the medium being responsible for the gas production.

Among the aerogenic strains, it is possible to correlate the carbohydrate fermentation pattern with urease production. The 7 strains which were urease positive had an identical fermentation pattern while strain 12, which is urease negative, had a pattern more closely resembling *P. multocida.*

The use of phenol red base browth in the Durham tube and phenol red semi-solid in other fermentation reactions allowed for a comparison of the 2 media. The phenol red base semi-solid was found to be a better medium for the slow-growing *Pasteurella.* The broth in the Durham tube often gave delayed results especially in the fermentation of xylose. The broth tube gave a false positive for 24-48 hours, but in 4-7 days the tube reverted to negative. The same strain when tested on semi-solid medium gave a negative reading for the 7 days. It was observed that the organism grew more slowly in broth than in the semi-solid medium.

The dye bacteriostasis experiment also resulted in a significant pattern of variation which is not apparent in Table 3. Table 5 presents a list of the strains which differed from the dye sensitivity pattern (Table 3) in their respective classes. Some of these variations seem to be more significant than random experimental errors. Strains 4, 6, 12, and 26 are all methyl violet positive although they are *P. multocida.*

Table 4. Gas production in aerogenic strains

No.	14	15	16	17	18	19	27	12
Strain No.	B92	B767	A8708	B704	B972	B1682	C566	CE49
Glucose	+/+*	+/+	+/+	+/-	+/+	+/+	+/+	+/+
Xylose	(-/-)	(-/-)	(-/-)	(-/-)	(-/-)	(-/-)	(-/-)	(-/-)
Mannitol	-/-	-/-	-/-	-/-	-/-	-/-	-/-	-/-
Lactose	-/-	-/-	-/-	-/-	-/-	-/-	-/-	-/-
Sucrose	+/-	+/-	+/-	+/-	+/+	+/+	+/+	-/-
Maltose	+/+	+/+	+/+	+/+	+/+	+/+	+/+	-/-
Sorbitol	-/-	-/-	-/-	-/-	-/-	-/-	-/-	+/+
Arabinose	-/-	-/-	-/-	-/-	-/-	-/-	-/-	-/-
Inositol	-/-	-/-	-/-	-/-	-/-	-/-	-/-	-/-
Dulcitol	-/-	-/-	-/-	-/-	-/-	-/-	-/-	-/-
Control	-/-	-/-	-/-	-/-	-/-	-/-	-/-	-/-
Urea	+	+	+	+	+	+	+	-

*Acid production/gas production.

Table 5. Dye bacteriostasis - variant strains

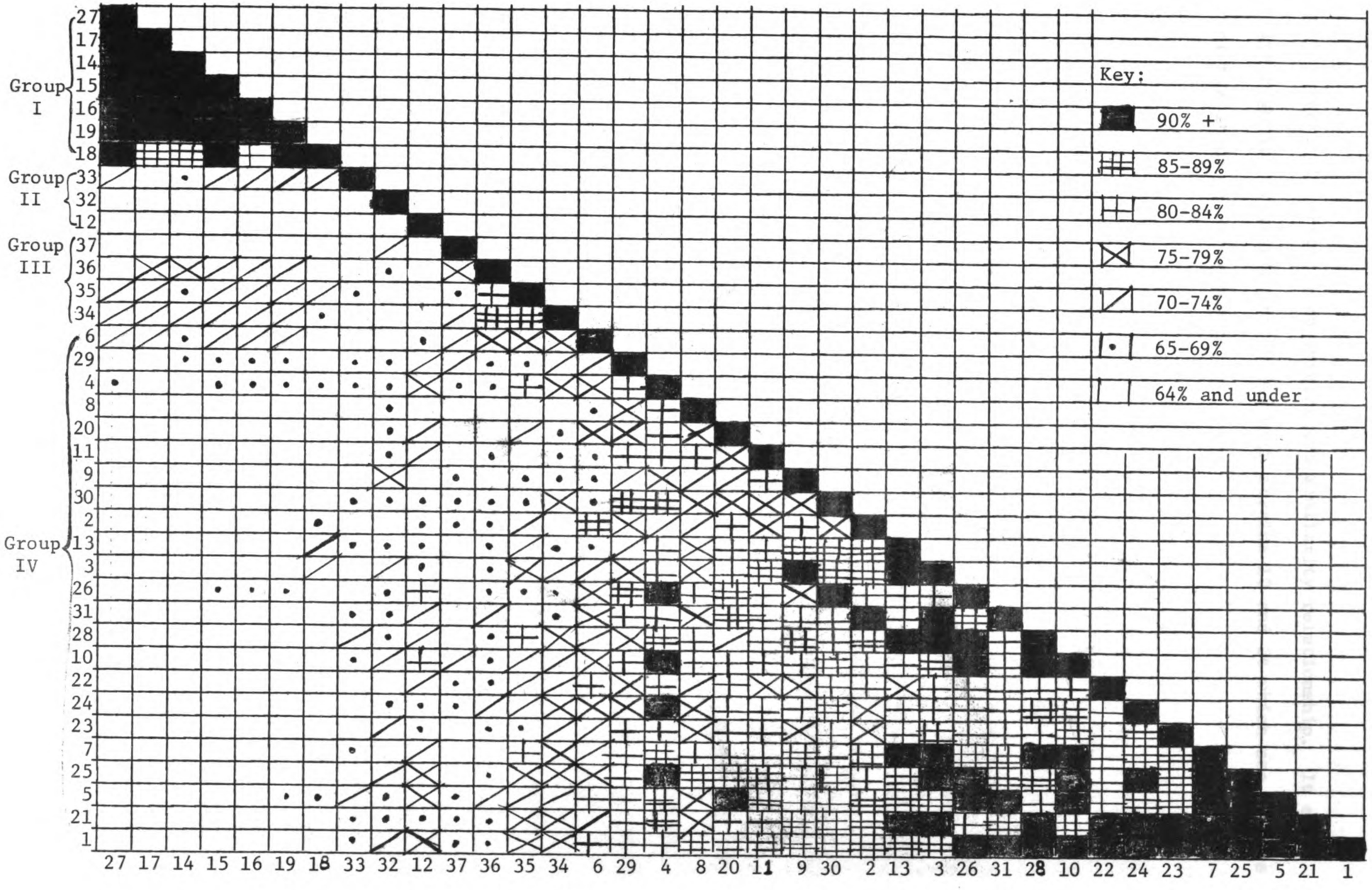
No.	2	4	6	10	12	13	17	18	26	27	30	36
Strain No.	CD 623	CC 1193	CE 28L	CC 971	CE 49	CC 1150	B704	B972	M17	C566	P1059	P309
Pyronin (1:500)	-	+	-	+	+	+	-	+	+	-	+	+
Methyl violet (1:100)	-	+	-	+	+	-	-	+	+	+	-	+
Basic fuschin (1:100)	-	-	-	-	-	+	+	+	-	+	+	-
Thionin (1:200)	-	+	+	+	+	+	-	+	+	-	+	+

Strain 27, urease positive gas producer of animal origin, was shown to have an identical pattern to other aerogenic strains of Dr. Weaver.

Due to the variation in each class, all the strains were compared to each other individually by calculating the percent similarities. The calculated S values range from 100% to 44%. Figure 1 is a similarity matrix based on this data with the strain order rearranged to form clusters of similar strains.

This matrix shows 4 groups. Group I is a cluster of 7 strains, including the 6 strains of *P.* "gas" (*multocida*?) and strain 27. This is a very homogeneous cluster with most S values 90% or over. The largest group, IV, which is composed of *P. multocida* of animal and human origin exhibits a great deal of heterogeneity. In this cluster, there is no separation of animal and human strains. In most cases, there is a high degree of similarity among them. However, there are some strains near the edge of the cluster which possess a low similarity. Strains 4 and 6 show a greater similarity to each other and strain 26 than the rest of the group. In general, the strains showing the lowest percent similarities are those from canine or feline origin or local infections of man. A simple relationship with this dissimilar group cannot be assumed because these strains often differ markedly from each other. Between Groups I and IV are the 2 intermediary groups. Group II is the 3 unrelated strains of *P. hemolytica*, *P. ureae*, and strain 12. Group III is composed of the 4 strains of *P. pneumotropica*.

The similarity matrix shows no cross relationship between Groups I and IV. A greater degree of similarity is seen between Groups I and III within a range of 70-79%. Group I exhibits little similarity to strain 12 which is the other aerogenic strain, but this group does show a similarity of 70-74% to *P. ureae* which is the other urease positive strain. It is unrelated to *P. hemolytica*.


Group I
27
17
14
15
16
19
18
Group II
33
32
12
Group III
37
36
35
34
Group IV
6
29
4
8
20
11
9
30
2
13
3
26
31
28
10
22
24
23
7
25
5
21
1
27 17 14 15 16 19 18 33 32 12 37 36 35 34 6 29 4 8 20 11 9 30 2 13 3 26 31 28 10 22 24 23 7 25 5 21 1
Key:
90% +
85-89%
80-84%
75-79%
70-74%
65-69%
64% and under

Strain 12 provides an interesting similarity relationship. It shows its highest correlation of 80-84% with strains 10 and 26 which are members of the group of *P. multocida*.

DISCUSSION

In this study, 37 strains of *Pasteurella* which were initially grouped by species origin and disease process were analyzed and compared to each other by biochemical reactions and other tests. Of special interest in this study were the strains which were found to be aerogenic. Most of the cultures were in the rough or blue form. There were some iridescent variants, but no attempt was made to eliminate them because virulence testing was not being carried out. It has been observed that colonial variation does not significantly affect certain biochemical characteristics such as indole production or carbohydrate fermentation (18).

Using the Durham tube to observe gas production, 8 of the 37 strains were shown to be aerogenic (Table 4). In all cases, the gas produced was in small quantities. Six of these 8 strains had been reported in the literature as positive for gas production from glucose, but the presence or absence of gas production with other sugars was not noted (56). It was not possible to repeat this gas production from glucose in the case of 1 strain, B-704. However, this strain was observed to produce gas from maltose. The other 5 strains also produced gas from maltose and in 3 cases from sucrose.

In addition to these 6 strains, 2 other strains were observed to be aerogenic. C-566, a canine isolate, produced gas from glucose, maltose, and sucrose, while CE-49, a human strain, produced gas from glucose and sorbitol. The production of gas from different carbohydrates by a single strain of *Pasteurella* has been previously reported. Talbot and Sneath

(54) isolated a strain of *P. multocida* from a dog which produced small quantities of gas from sucrose, maltose, mannose, and trehalose.

The 8 aerogenic strains studied did not ferment the same carbohydrates. A direct correlation between carbohydrate fermentation and urease production was observed. All the urease positive strains fermented glucose, sucrose and maltose and were negative for sorbitol, while the one urease negative strain fermented glucose and sorbitol but did not ferment sucrose and maltose.

There were, therefore, 7 closely related aerogenic strains corresponding in biochemical reaction to *P.* "gas" (*multocida*?). The other aerogenic strain was unrelated.

In addition to the biochemical reactions and other tests which are used to classify *Pasteurella*, an attempt was made to characterize this species by dye bacteriostasis. Rosenbusch and Merchant (46) tried unsuccessfully to do this in 1939. With a modified version of the dye sensitivity disc testing method of Pickett, Nelson, Hoyt and Eisenstein (41) a repeatable pattern of dye sensitivity was obtained. *Pasteurella multocida*, *P. pneumotropica*, and *P.* "gas" (*multocida*?) had unique patterns. The strain of *P. hemolytica* studied and *P. multocida* could not be distinguished by this method.

If the dye sensitivity testing method proved consistent with a large number of strains, it might become a useful technique to use with other tests to subgroup *P. multocida* into a series of biotypes similar to the work done on *Brucella*. An indication of this possibility was given when 3 strains of *P. multocida* which varied in a consistent manner from the "typical" dye sensitivity pattern of the other strains proved to be more closely related to each other than the majority of other *P. multocida* strains when analyzed in the similarity matrix (Figure 1).

The aerogenic strains of *Pasteurella* could not be adequately compared to each other by using tabulated charts of biochemical data. An attempt was made to analyze the data according to the method used in Smith and Thal (50). They manually constructed a similarity matrix comparing 25 strains of *Pasteurella* of various species. They found this method to be very useful for comparing small numbers of strains. If more extensive studies are undertaken, computer analysis is necessary.

With this method, the 8 aerogenic strains could be compared individually to each other and to the other strains tested. All the strains rearranged in the similarity matrix fell into 4 broad groups. Group I consisted of 6 aerogenic strains of Dr. Weaver and strain 27. Group II consisted of 3 unrelated strains of *P. hemolytica*, *P. ureae*, and strain 12. Group III was composed of strains of *P. pneumotropica*. Group IV was made up of human and animal strains of *P. multocida*.

The validity of the similarity matrix constructed was confirmed by a comparison of similarities between species established by this matrix and the one constructed by Smith and Thal (50). The percent similarity of *P. multocida* and *P. pneumotropica*, *P. multocida* and *P. hemolytica*, *P. ureae* and *P. multocida* are generally in agreement between the 2 matrices. When a slight variation occurred, the percent similarity of the experimental matrix is lower than the percent similarity contained in the matrix of Smith and Thal.

When the Group I strains are compared to the other groups in the experimental matrix, it was found to be the most closely related to *P. pneumotropica* and *P. ureae*. It is felt that the similarity is probably closer to *P. pneumotropica* strains than *P. ureae*. The limited number of *P. pneumotropica* used in this study had some irregular carbohydrate fermentation reactions, and proved to be ornithine decarboxylase positive

even though this species is considered to be typically negative for this test. If a typical strain of *P. pneumotropica* (50) was compared to the *P.* "gas" (*multocida*?) strains, the S values would be in the range of 90%. *Pasteurella ureae* is also less likely to be the most closely related species because *P. ureae* and *P.* "gas" (*multocida*?) differ in indole reaction which is considered a key reaction of the *Pasteurella* species in classical taxonomy.

The Group I aerogenic strains differed completely from strain 12, the urease negative aerogenic strain. Strain 12 showed its greatest similarity to some of the *P. multocida* strains. It was not similar to all of the strains. Group IV varied considerably within the group.

There have been repeated attempts to correlate the variations of *P. multocida* with species differences of host or some other traceable variable. In this matrix, it was the canine, feline and local infections of man which were the most dissimilar and were found on the fringes of the group. Canine and feline strains have been observed to differ from the species as a whole (48). The subgrouping of *P. multocida* is probably highly complex. Strains of Group IV dissimilar to the more common varieties of the group are often dissimilar in varying degrees to each other.

In summary, the aerogenic strains of Dr. Weaver and strain 27 seem most closely related to *P. pneumotropica*. Strain 12 seems to be the most closely related to *P. multocida*. It would appear that the best way in which to taxonomically rank these strains are as varieties of the previously established species. To establish new species would probably only complicate an already complex situation. It must be remembered that any bacterial taxonomic classification system is at best a series of approximate points on a scale of natural progression which is constantly adapting and making biologic adjustment.

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