Numerical Taxonomy of *Haemophilus* and Related Bacteria

P. H. A. SNEATH and R. JOHNSON

*Medical Research Council Microbial Systematics Unit, University of Leicester, Leicester, England, and
Computer Trials Laboratory, Central Public Health Laboratory, Colindale, London NW9, England*

A numerical taxonomic study of named strains of *Haemophilus* Winslow et al., *Actinobacillus* Brumpt, and *Pasteurella* Trevisan was made using 134 characters covering a wide range of properties. The strains fell into four main clusters. Three of these represented *Haemophilus*. Cluster 1 contained strains requiring X factor and usually also V factor and corresponded to *H. influenzae* (Lehmann and Neumann) Winslow et al. and closely allied organisms. Cluster 2 contained strains that required only V factor and corresponded to *H. parainfluenzae* Rivers and similar organisms. Cluster 3 contained strains with a requirement for a raised CO₂ tension and contained *H. aphrophilus* Khairat and *H. paraphrophilus* Zinnemann. Cluster 4 contained strains of *Actinobacillus* and *Pasteurella* but not *Yersinia* van Loghem. The three genera *Actinobacillus*, *Pasteurella*, and *Haemophilus* have a close phenetic relationship and are facultative anaerobes that can acidify glucose fermentatively. They might best be separated from the other genera of the family *Brucellaceae* Breed et al.

The genus *Haemophilus* Winslow et al. 1917, although of considerable medical importance, has received relatively little detailed study, as shown by the paucity of comparative data in works such as the 7th edition of *Bergey's Manual* (7). Some species once included in the genus have been segregated as a separate genus, *Bordetella*; the remainder have been studied mainly with respect to rather few properties, notably growth requirements and hemolysis. The taxonomic structure within the genus, and its relation to other genera, is uncertain, and we therefore present this brief report in the hope that it will stimulate further comparative work on the group.

The 7th edition of *Bergey's Manual* contains 15 species of *Haemophilus*. Several of these (*H. citreus*, *H. influenzae-murium*, *H. ovis*, and *H. putoriorum*) are very poorly known, and authentic strains do not appear to be available, although attempts are being made to reisolate some of them (4). Certain other species assigned to *Haemophilus* were noted in an earlier edition of *Bergey's Manual* (6) or in *Index Bergeyana* (8) under *Haemophilus* or *Hemophilus*; these are even more poorly known and include *H. anatipestifer*, *H. cuniculi*, *H. meningitidis*, and *H. muris*. Species described more recently include *H. paraphrophilus* Zinnemann et al. 1968 and *H. paraphrohaemolyticus*

Zinnemann et al. 1971, which are similar in many ways to *H. aphrophilus* Khairat 1940. *H. agni* Kennedy et al. 1958, which causes a septicaemia of lambs, also appears to resemble *H. aphrophilus*, and similar organisms have been isolated from meningoencephalitis of cattle (25, 46).

Haemophilus pleuropneumoniae Shope 1963, a very pleomorphic, V-requiring organism causing pleuropneumonia of swine, is similar to the strains of influenza bacilli from swine that require V factor only (47), and thus it is probably similar to *H. parainfluenzae* and to the recently described species *H. paragallinarum* Biberstein and White 1969 and *H. parasuis* Biberstein and White 1969. Another new species, associated with an encephalitis of cattle, was described by W. E. Baillie (Ph.D. thesis, Kansas State Univ., Manhattan, 1969) as *Haemophilus somnus*; however, this name was not effectively published. The taxonomic position of this organism is uncertain because, although it resembles *H. influenzae* in appearing to require both X and V factors and in being indole positive, it resembles *H. parainfluenzae* in being oxidase positive and urease negative (see results reported below).

Haemophilus ducreyi (apparently illegitimate; the correct specific epithet according to Buchanan, Holt, and Lessel (8) is *ulceris-*

cancrosi) is, on clinical grounds, a distinct species, and so is *H. piscium* Snieszko et al. 1950, although the latter may well belong in some other genus because it has a number of properties not found in other species of *Haemophilus* (7). *H. vaginalis* Gardner and Dukes 1955 is now usually considered to belong to some other genus (13, 41, 67).

Type strains are available for only a few of these species, and this has been a major limitation of the present study. There is no type or neotype strain of the type species *H. influenzae* (Lehmann and Neumann 1896) Winslow et al. 1917, and although it must be admitted that the specific identity of the original strains would be hard to establish, the organism now given that name is well known, and numerous strains can be accepted as authentic.

MATERIALS AND METHODS

In the course of an investigation of the genus *Bordetella* and related genera (R. Johnson, Ph.D. thesis, Univ. of Leicester, England, 1972; reference 22), 29 strains of *Haemophilus*, representing all the species readily available to us, were studied (Table 1). It should be noted that the specific identity of some strains is uncertain (see Discussion) and that the strains are referred to here by the names under which they were received. It was noted that strains of species of *Actinobacillus* and *Pasteurella* came unexpectedly close to the *Haemophilus* strains, and results on these strains are therefore included in this report. The strains were examined for a wide range of characters, including morphology, colonial properties, growth requirements, resistance to various chemicals, attack on carbohydrates, and physiological and biochemical tests. X and V factors were added to media at concentrations of 2.5 mg/liter, or were supplied as disks (Oxoid Ltd.) containing haemin (10 µg), nicotinamide adenine dinucleotide (10 µg), or both, placed on the surface of inoculated agar plates of medium lacking these factors, and incubated in air at 35 °C. Details of the basal medium and methods are given by Johnson (Ph.D. thesis, Univ. of Leicester, England, 1972) and Johnson and Sneath (22). When a medium of low salt concentration was required, it was made up from its constituents omitting NaCl and contained only about 0.04% NaCl derived from added serum. Carbohydrate tests could be satisfactorily made in the medium of Hugh and Leifson (19) with 30 mg of bromothymol blue per liter as indicator if 2.5 mg of haemin and of nicotinamide adenine dinucleotide per liter (sterilized by filtration) were added after autoclaving.

The results were coded as 134 characters; almost all were presence-absence characters, but a few quantitative characters were coded 0, 1, 2, or 3. The amount of experimental error, although not examined in detail for the *Haemophilus* strains, is believed to be only a few percent (see 56), and this is supported by

the high level of similarity of 92% between J73 and J74, which are both derived from one strain, Pittman 180a.

Several different numerical taxonomic methods were employed. The findings described here are based on computations using the similarity coefficient of Gower (16), including negative matches (this is almost equivalent to a simple matching coefficient [see reference 59] when almost all characters are qualitative, as in the present study), followed by average-linkage cluster analysis (UPGMA, see reference 59). The other methods included computations omitting 22 carbohydrate fermentations, the coefficient S_J (which excludes negative matches), the vigor coefficient, the pattern similarity coefficient S_P , and single-linkage clustering (see references 53 and 59). However, results from all of these on the *Haemophilus* strains were very similar, so that only a few points of interest revealed by them are mentioned.

RESULTS

The strains of *Haemophilus* fell into three fairly well-separated clusters (Fig. 1 and 2). These three groups formed at quite high S levels, over 82%, and they remained distinct until the similarity level fell to about 78%. The fourth cluster contained the strains of *Actinobacillus* and *Pasteurella*. The properties of the four groups are shown in Table 2, from which the hypothetical median organisms (32) can be calculated. Although there was no clear separation between *Actinobacillus* and *Pasteurella*, the results for the two genera have for convenience been tabulated separately.

The four groups share the following salient and almost invariant properties: they are small, gram-negative rods or coccobacilli, nonmotile, often pleomorphic with filamentous forms, that are facultative anaerobes and ferment glucose anaerobically with the production of acid but no gas. They grow on blood agar (although *Haemophilus* strains grow poorly unless supplemented with X or V factors or in raised CO₂ concentration when required), giving smooth, round, low, convex, or flattened colonies that are transparent or translucent, commonly showing some hemolysis on horse blood agar and occasionally slight greening. They are methyl red negative, Voges-Proskauer negative, and nonproteolytic, but are H₂S positive and phosphatase positive.

Cluster 1 contained all the strains of *H. influenzae*. The strains of *H. aegyptius* and *H. haemoglobinophilus* came into this group, and they may perhaps represent subgroups within this cluster because they link to the others at lower similarity levels than most of the strains in the cluster. However, the two strains of *H. aegyptius* are believed to derive from a single

original isolate. The strain received as *H. haemolyticus* also fell here.

All the strains of this cluster required X factor, and all except the two strains of *H.*

haemoglobinophilus also required V factor. None of the strains required added CO₂ for good growth. On horse blood agar containing added V factor, or on chocolate agar (heated

TABLE 1. *Strains studied*

Cluster	Strain no. in this survey	Name as received	Source and collection no. ^a	Comments
1	J 73	<i>Haemophilus aegyptius</i>	NCTC 8502	Proposed working type ^b , Pittman 180a
	J 74	<i>H. aegyptius</i>	ATCC 11116	Another culture of Pittman 180a
	J 78	<i>H. haemoglobinophilus</i>	NCTC 1659	Co-type ^a
	J 79	<i>H. haemoglobinophilus</i>	Zinnemann	
	J 80	<i>H. haemolyticus</i>	NCTC 8479	Proposed working type, ^b but see Discussion
	J 83	<i>H. influenzae</i>	NCTC 4560	Proposed working type ^b
	J 86	<i>H. influenzae</i>	ATCC 9006	Pittman serotype a
	J 87	<i>H. influenzae</i>	NCTC 8645	Pittman serotype a
	J 88	<i>H. influenzae</i>	NCTC 7279	Pittman serotype b
	J 89	<i>H. influenzae</i>	NCTC 8496	Pittman serotype c
	J 90	<i>H. influenzae</i>	NCTC 8470	Pittman serotype d
	J 91	<i>H. influenzae</i>	NCTC 8472	Pittman serotype e
	J 92	<i>H. influenzae</i>	NCTC 7918	Pittman serotype f
	J 182	<i>Haemophilus</i> sp.	PHLL	Recent isolate from human respiratory tract
	J 183	<i>Haemophilus</i> sp.	PHLL	Recent isolate from human respiratory tract
2	J 93	<i>Haemophilus suis</i>	NCTC 4557	Co-type, but see Discussion ^b
	J 94	<i>H. suis</i>	CAPM 5111	Pig lung, Czechoslovakia
	J 95	<i>H. suis</i>	CAPM 5113	Pig lung, Czechoslovakia
	J 99	<i>H. parainfluenzae</i>	NCTC 4101	
	J 100	<i>H. parainfluenzae</i>	ATCC 7901	
	J 101	<i>H. parainfluenzae</i>	ATCC 9796	
	J 102	<i>H. gallinarum</i>	CAPM 5015	Fowl, Canada, 1962
	J 103	<i>H. gallinarum</i>	CAPM 5066	Fowl, Czechoslovakia
	J 104	<i>H. gallinarum</i>	CAPM 5080	Fowl, Czechoslovakia
	J 108	<i>H. parasuis</i>	CAPM 5114	
	J 125	<i>Haemophilus</i> sp.	CAPM P392	
3	J 75	<i>Haemophilus aphrophilus</i>	NCTC 5886	Holotype ^b
	J 120	<i>H. aphrophilus</i>	NCTC 5906	A co-type (see 4)
	J 121	<i>H. paraphrophilus</i>	NCTC 10557	Holotype (65)
4	J 19	<i>Actinobacillus equuli</i>	NCTC 8529	Proposed working type ^b
	J 20	<i>A. lignieresii</i>	NCTC 4975	
	J 21	<i>A. lignieresii</i>	NCTC 4976	Proposed working type ^b
	J 167	<i>Pasteurella multocida</i>	NCTC 3195	Proposed working type ^b
	J 169	<i>P. pneumotropica</i>	NCTC 8141	
	J 170	<i>P. ureae</i>	NCTC 10222	
	J 171	<i>P. haemolytica</i>	NCTC 10365	Type A of Smith (50, 51)
	J 245	<i>P. piscicida</i>	ATCC 17911	Co-type (21)
	J 246	<i>P. ureae</i>	PHLL	

^a Abbreviations: ATCC, American Type Culture Collection, Rockville, Md.; CAPM, Collection of Animal Pathogenic Microorganisms, Brno-Medlánky, Czechoslovakia; NCTC, National Collection of Type Cultures, London; PHLL, Public Health Laboratory, Leicester, England; Zinnemann, K. Zinnemann, Department of Bacteriology, School of Medicine, Leeds, England.

^b From Sneath and Skerman (57).

blood agar), and after 48 h at 35 C the colonies were 0.5 to 2.0-mm in diameter, circular, low conical or flat topped, often with a central umbo and bevelled edge. The edge was entire or occasionally slightly lobed and often with radial striations on the bevel. The surface was smooth or undulant, and shiny. The growth was colorless or slightly gray, translucent, and butyrous. There was no pitting around the colony (i.e., the edge was not sunken below the agar surface). On blood agar, beta hemolysis was scanty or absent (except for the hemolytic strain J80), but there was often some green discoloration of the medium. On blood agar without V factor, colonies were 0.3 to 0.5-mm in diameter, low convex or only slightly

flattened, and showed marked satellitism around V factor disks or plate contaminants (except for strains J78 and J79). Cultures had a pronounced smell of amines, similar to putrescine.

Cluster 2 contained the strains received as *H. parainfluenzae*, *H. suis*, and *H. gallinarum* as well as one unidentified strain. All of these required V factor but not X factor. They did not require added CO₂ for good growth. On horse blood agar with V factor, or on chocolate agar, colonies were similar to those of strains of cluster 1 except in the following. They were seldom umbonate, and the edge was not bevelled and was without radial striations. The surface was somewhat smoother, and the

FIG. 1. Shaded similarity matrix of strains of *Actinobacillus*, *Haemophilus* and *Pasteurella* (Gower's similarity coefficient including negative matches).

FIG. 2. Phenogram of strains of *Actinobacillus*, *Haemophilus*, and *Pasteurella* (UPGMA clustering of similarities from Fig. 1). The co-phenetic correlation coefficient is 0.84.

growth was sometimes semiopaque, commonly tinged pale yellow. Hemolysis and green discoloration was still less marked, but satellitism and the smell of amines were both present.

Cluster 3 contained *H. aphrophilus* and *H. paraphrophilus*. The two strains of the former required X but not V factor (though *H. aphrophilus* mutates regularly to X-independence [see reference 5]), whereas the latter required V but not X. Nevertheless, the two species are otherwise very similar. All three strains require added CO₂ for good growth even in the presence of X plus V factor (the CO₂ concentration was 5% in air; the basal medium contained a low level of sodium chloride, about 0.04%). On horse blood agar with V factor, or

on chocolate agar, incubated in 5% CO₂, the colonies were similar to those of cluster 1 except in the following ways. They were high convex, not flattened or umbonate, with an entire edge without radial strictions or bevel, and the growth was translucent, gray or yellowish, and that of strain J121 was viscid. Hemolysis was slight, though on long incubation J121 gave partial beta hemolysis with some green discoloration. In the absence of added CO₂, colonies were minute, and satellitism was only shown by J121. The cultures did not smell of amines. The only significant discrepancies from the original species descriptions (27, 65) was that strains J75 and J120 acidified fructose and galactose.

TABLE 2. *Characters of groups as percentage of positive reactions*^a

Character	<i>Actino- bacillus</i> (3) ^b	<i>Pasteurella</i> (6)	<i>Haemophilus influenzae</i> group (15)	<i>Haemophilus parainfluenzae</i> group (11)	<i>Haemophilus aphrophilus</i> group (3)
Gram reaction (2 days)	0 ^c	0	0	0	0
Retention of methyl violet (2 days)	0	50	0	0	0
Chains or filaments present (2 days)	100	33	73	100	33
Pleomorphism (2 days)	100	33	73	100	33
Cocci or coccobacilli (2 days)	100	33	0	0	33
Colony opaque	0	33	0	0	0
Colony viscid	100	0	0	0	0
Pellicle on broth	67	33	0	0	0
Diffusible brown pigment	0	0	0	0	0
Pigment from tyrosine	0	0	0	0	0
Dextran or levan production	0	0	0	0	0
Growth anaerobically	100	100	100	100	100
Growth anerobically with nitrate	100	100	100	100	100
CO ₂ requirement	0	0	0	0	100
X factor requirement	0	0	100	0	67
V factor requirement	0	0	87	100	33
Growth on nutrient agar	100	100	0	0	0
Growth on blood agar	100	100	100	100	100
Hemolysis of horse red cells	0	67 ^d	47	27	0
Hemolysis of sheep red cells	0	0	7	0	0
Motility (14 days)	0	0	0	0	0
Growth on 5% bile salts	100	67	53	55	100
Growth on 10% bile salts	0	17	7	27	0
Growth on 40% bile salts	0	0	0	0	0
Growth on 0% NaCl	100	67	100	100	100
Growth on 3% NaCl	67	67	0	55	0
Growth on 4.5% NaCl	0	0	0	0	0
Growth on 6% NaCl	0	0	0	0	0
Growth on 7.5% NaCl	0	0	0	0	0
Growth on 9% NaCl	0	0	0	0	0
Growth on 0.1% phenol agar	0	50	7	0	0
Growth on 0.2% phenol agar	0	0	0	0	0
Growth on 0.3% phenol agar	0	0	0	0	0
Growth on 0.4% phenol agar	0	0	0	0	0
Growth on 0.5% phenol agar	0	0	0	0	0
Growth on 320 mg of potassium tellurite per liter	0	0	13	0	0
Growth on 75 mg of sodium azide per liter	100	100	100	100	100
Growth on 0.5% thalious acetate	0	0	13	9	0
Growth on 12.5 mg of M and B 938 per liter	0	67	13	82	0
Growth on 0.5% sodium nitrite	100	100	13	45	0
Growth at 5 C (10 days)	0	0	0	0	0
Growth at 10 C (10 days)	0	0	0	9	0
Growth at 15 C (10 days)	100	33	0	18	0
Growth at 25 C (10 days)	100	100	100	91 ^d	100 ^d
Growth at 35 C (10 days)	100	100	100	100	100
Growth at 37 C (10 days)	100	83	100	100	100
Growth at 44 C (10 days)	0	0	7	64	0
Sensitivity to penicillin	100	100	93	64	100

Cluster 4 contained the strains of *Actinobacillus* and *Pasteurella*, which are associated at a level of over 75% *S*. Although the strains of *Actinobacillus equuli* and *A. lignieresii* fall

together (and are indeed extremely similar), there is here no strong evidence that *Actinobacillus* forms a genus distinct from *Pasteurella* (see Discussion). They did not require X or V

TABLE 2—Continued

Character	<i>Actino- bacillus</i> (3) ^b	<i>Pasteurella</i> (6)	<i>Haemophilus</i> <i>influenzae</i> group (15)	<i>Haemophilus</i> <i>parainfluenzae</i> group (11)	<i>Haemophilus</i> <i>aphrophilus</i> group (3)
Sensitivity to streptomycin	100	100	100	100	100
Sensitivity to chloramphenicol	100	100	100	91	100
Sensitivity to aureomycin	100	100	100	100	100
Sensitivity to terramycin	100	100	100	100	100
Sensitivity to erythromycin	100	100	100	73	33
Sensitivity to tetracycline	100	100	100	91	100
Sensitivity to sulfonamide	100	50	27	0	100
Sensitivity to neomycin	100	100	100	100	100
Sensitivity to novobiocin	100	100	100	100	100
Sensitivity to oleandomycin	100	100	100	100	100
Sensitivity to nitrofurantoin	100	100	100	100	100
Sensitivity to bacitracin	0	0	0	0	0
Sensitivity to Bactrim Roche	100	100	7	9	33
Sensitivity to 0/129	67	83	33	0	67
Sensitivity to thionine	0	17	27	0	0
Sensitivity to basic fuchsin	67	67	93	9	0
Sensitivity to methyl violet	100	50	93	36	0
Sensitivity to safranin	33	17	40	45	0
Sensitivity to pylonin	100	50	53	9	0
Heat resistance: 56 C, 5 min	33	67	53	91	33
Heat resistance: 56 C, 10 min	0	33	33	82	33
Heat resistance: 56 C, 15 min	0	33	20	64	0
Heat resistance: 56 C, 20 min	0	0	13	36	0
Catalase (2 days)	100	83	100	91	0
Peroxidase (2 days)	100	67	0	9	0
Oxidase (2 days)	100	100	7	73	0
H ₂ S production	100	100	100	100	100
Phosphatase (5 days)	100	100	80	100	100
Gelatin hydrolysis	0	0	0	0	0
Starch hydrolysis	100	100	33	100 ^d	0
Turbidity from egg yolk	0	0	0	0	0
Casein hydrolysis	0	0	0	0	0
Aesculin hydrolysis	0	0	7	0	0
Tetrazolium reduction	100	100	100	100	100
Indole production	0	0	53	0	0
Ammonia from peptone	100 ^d	67	80	73 ^d	100 ^d
Urease (1 h)	100	50	87	0	0
Arginine hydrolysis (1 day)	0	50	7	0	0
Nitrate reduction	100	50	67	82	100
Deoxyribonuclease	0	0	0	0	0
Methyl red	0	17	20	55	0
Voges Proskauer	0	0	0	0	0
Tyrosine hydrolysis	0	0	0	0	0
Xanthine hydrolysis	0	0	0	0	0
Phenylalanine deaminase	0	0	0	0	0
Tween 20 hydrolysis	0	0	0	0	0
Tween 40 hydrolysis	0	0	0	0	0
Tween 60 hydrolysis	0	0	0	0	0
Tween 80 hydrolysis	0	0	0	0	0
Clearing of Tween 80	33	0	0	0	0
Acid aerobically from 1% glucose	100	100	100	100	100
Acid anaerobically from 1% glucose	100	100	100	100	100
Acid aerobically from 10% glucose	100	100	0	0	100
Acid aerobically from 1% lactose	0	0	0	0	100
Acid aerobically from 10% lactose	0	0	0	0	100
Alkali aerobically from 1% lactose	0	33	0	0	0
Acid aerobically from xylose	100	33	40	9	0
Acid aerobically from salicin	0	0	0	0	0
Acid aerobically from raffinose	0	17	0	0	0
Acid aerobically from methyl-D-glucoside	0	0	0	0	0

TABLE 2—Continued

Character	<i>Actinobacillus</i> (3) ^b	<i>Pasteurella</i> (6)	<i>Haemophilus influenzae</i> group (15)	<i>Haemophilus parainfluenzae</i> group (11)	<i>Haemophilus aphrophilus</i> group (3)
Acid aerobically from ribose	100	67	27	55	100
Acid aerobically from rhamnose	0	0	0	0	0
Acid aerobically from mannose	100	100	0	73	100
Acid aerobically from inositol	0	33	0	0	0
Acid aerobically from cellobiose	0	0	0	0	100
Acid aerobically from fructose	100	50	20	100	100
Acid aerobically from L(+)-arabinose	100	17	0	55	0
Acid aerobically from dextrin	100	83	87	91	67
Acid aerobically from sorbitol	0	50	0	0	0
Acid aerobically from galactose	100	100	60	73	100
Acid aerobically from maltose	100	100	60	55	100
Acid aerobically from sucrose	0	100	0	82	100
Acid aerobically from mannitol	100	83	0	9	0
Acid aerobically from glycerol	0	33	0	18	100
Acid aerobically from glucosamine	0	83	100	100	100
Acid aerobically from inulin	0	0	7	55	100
Acid aerobically from adonitol	0	0	0	0	0

^a All strains grew on 0.5% NaCl, at 30 °C, and in the presence of optochin, and none produced hydrogen cyanide or acid from dulcitol.

^b Number of strains studied indicated in parentheses.

^c Symbols: figures indicate the percentage of strains positive within 7 days (unless otherwise noted).

^d Weak but definite reactions.

factors or a raised CO₂ tension for growth. On horse blood agar and chocolate agar the colonial morphology was variable as would be expected, but the colonies were all large (1.5–3.0 mm after 48 h at 35 °C), they were usually low conical, smooth, shiny or matte, sometimes umbonate, and had no bevelled edge (except J169). Growth was grayish and transparent to semiopaque. J20 and J21 showed slight but definite pitting, with the edge sunken below the surface of the medium, and, together with J19, gave viscid colonies. Hemolysis was often noticeable, usually beta in type with some green or brown discoloration. There was no satellitism, and cultures did not have the characteristic smell of cluster 1 strains.

Table 3 shows the mean inter- and intragroup *S* values for these four clusters. Included in the tables are the values for "vigor" (53), which is one index of metabolic activity. It is seen that the strains of the *Actinobacillus-Pasteurella* cluster are appreciably more active than those of the *Haemophilus* clusters. The vigor differences between the *Haemophilus* clusters are not statistically significant by the *t* test at the 5% confidence level, with one exception: strains of cluster 1 are significantly less active than those of cluster 2. Because the vigor differences between the *Haemophilus* clusters are so small, the pattern similarity coefficient *S_P* (that provides some correction for discrepancies in

TABLE 3. Mean intergroup and intragroup percentage *S* values, and vigor, with their standard deviations

Clusters	Percentage <i>S</i> values				
	1 ^a	2	3	4	Vigor
1	85.43 (3.95)				0.394 (0.028)
2	78.39 (3.49)	85.81 (3.87)			0.430 (0.026)
3	75.09 (3.20)	78.39 (3.49)	91.20 (2.29)		0.414 (0.034)
4	73.91 (3.61)	74.93 (2.41)	71.23 (2.90)	80.87 (5.09)	0.485 (0.020)

^a Clusters.

vigor, growth rate, incubation temperature, and the like, see reference 59) gave results almost identical to those shown in Fig. 1 and 2. The three *Haemophilus* clusters were unchanged except for minor rearrangement within them, and they appeared somewhat more compact and distinct. The *Actinobacillus-Pasteurella* cluster was still closer to the *Haemophilus* clusters; within it there was slight rearrangement, but there was one aberrant result—strain J167 (*P. multocida*) appeared outside the group among a residue of poorly characterized organisms peripheral to *Yersinia* strains that are not

considered in the present report. Also, a strain J153 (=ATCC 11479) received by us under the name of *Noguchia granulosis*, but possibly not an authentic strain (22), became a satellite of the *Actinobacillus-Pasteurella-Haemophilus* complex.

Omission of 22 carbohydrate fermentations (but still using Gower's coefficient and UPGMA) again made very little difference. The *Haemophilus* clusters were practically unchanged. Cluster 4 showed some rearrangement, but the only notable feature was that a strain of *Pasteurella haemolytica* type T (50, 51), J172 (=NCTC 10371), moved into this cluster from a position peripheral to *Yersinia*. Again *Noguchia granulosis* was a satellite of the *Actinobacillus-Haemophilus-Pasteurella* complex. The major division of this complex from other genera of *Brucellaceae* was maintained (22).

The differences with change of the computing method mentioned above are relatively small, and a glance at the strains that moved in

or out of the four clusters shows them to be single representatives of bacterial species; they also may well be peripheral to the main groups studied here or intermediate between them. They are thus quite likely to show some instability.

The genera *Actinobacillus*, *Haemophilus*, and *Pasteurella* share a number of characters. They are non motile; they grow anaerobically and produce acid anaerobically from 1% glucose; they usually grow on agar without NaCl but do not grow on 4.5% NaCl agar; they are phosphatase positive, produce H₂S readily, and seldom hydrolyze Tween 80 or arginine. Closely related genera such as *Alcaligenes*, *Bordetella*, *Brucella*, and *Moraxella* differ in most of these characters, but the differences from *Yersinia* are fewer. However *Yersinia* strains generally grow on 4.5% NaCl, hydrolyze arginine, and are phosphatase negative. In addition *Yersinia* strains produce a characteristic clearing in plates containing 1% Tween 80

TABLE 4. Differential features of clusters

Characters	1 <i>Haemophilus</i> <i>influenzae</i> group (cluster 1)	2 <i>Haemophilus</i> <i>parainfluenzae</i> group (cluster 2)	3 <i>Haemophilus</i> <i>aphrophilus</i> group (cluster 3)	4 <i>Actino-</i> <i>bacillus-</i> <i>Pasteurella</i> group (cluster 4)
Growth on nutrient agar in air	— ^a	—	—	+
X factor requirement	+	—	v	—
V factor requirement	v	+	v	—
CO ₂ requirement on low NaCl medium	—	—	+	—
Sensitivity to basic fuchsin, 1/200,000 ^b	+	—	—	v
Sensitivity to 0/129 ^c	v	—	v	+
Sensitivity to M&B 938 ^d	+	—	+	v
Catalase	+	+	—	+
Oxidase ^e	—	v	—	+
Urease	+	—	—	v
Nitrate reduction	v	+	+	v
Indole production	v	—	—	—
Starch hydrolysis	v	v	—	+
Acid from 1% lactose	—	—	+	—
Acid from 1% fructose	—	+	+	v
Acid from 1% arabinose	—	v	—	v
Acid from 1% cellobiose	—	—	+	—
Acid from 1% sucrose	—	+	+	v
Acid from 1% mannitol	—	—	—	+
Acid from 10% glucose	—	—	+	+

^a Symbols: +, more than 80% of strains positive within 7 days; —, less than 20% of strains positive within 7 days; v, between 20 and 80% of strains positive within 7 days.

^b Filter paper strip (Whatman no. 1), 0.5-cm wide soaked in 1/200,000 aqueous solution (5 µg/ml) and dried, incorporated into heated blood agar plates, and cross-streaked with organism.

^c Paper disk containing 40 µg of 2,4-diamino, 6,7 di-isopropyl pteridine.

^d Basal medium containing 12.5 mg/ml of 4:4' diamidinodiphenylamine dihydrochloride.

^e Method of Kovacs (28) read after 10 s.

that is not shown by organisms of the *Actinobacillus-Haemophilus-Pasteurella* group or by most of the other genera mentioned, although *Alcaligenes* and *Bordetella bronchiseptica* do show this clearing (22).

The features that distinguish the four clusters of the *Actinobacillus-Haemophilus-Pasteurella* group are shown in Table 4. Carbohydrate tests in the Hugh and Leifson medium were more sensitive than the method used for the computer analysis (in which indicator was added only after incubation because of the risk of inhibition of *Bordetella pertussis* strains in the other part of this survey). With Hugh and Leifson medium, all strains in clusters 2 and 3, but few strains of cluster 1, acidified mannose and galatose. Two-thirds of the strains in cluster 1 but only one-third of the strains of cluster 2 acidified xylose in this medium. Most strains of cluster 2 acidified raffinose in this medium. Fewer positives were obtained in this medium with dextrin than by the other technique. Otherwise the carbohydrate tests were in good agreement.

DISCUSSION

Haemophilus appears to be an acceptable genus because the three clusters of strains in this genus are quite similar, and they unite before being joined by the *Actinobacillus-Pasteurella* cluster. The three genera are evidently close, although there appears to be no critical evidence from genetics or serology to support this (23). They do not greatly differ in deoxyribonucleic acid base composition, having G/C ratios in the 35 to 45% range (17, 18, 44).

Even though it is difficult to base conclusions on the results of such a small number of strains, the data provide quite strong evidence against the separation of *Actinobacillus* and *Pasteurella* as distinct genera. It has been known for many years that *Actinobacillus lignieresii* and *Pasteurella multocida* are phenetically very similar (55), and more recently Mraz (33, 34, 35) has given further evidence of the close similarity of both A and T types of *P. haemolytica* to *A. lignieresii*. It may be noted that it is difficult to identify individual strains as to genus until after they have been identified to species. If after further study it is considered best to combine the genera, the name *Pasteurella* would apply, as it is the older name (8, 24).

There is certainly doubt about the generic identity of some of the strains we studied. However, the strains of *H. haemoglobinophilus*

required only X factor as expected (42), and one of them was a type strain. Similarly, *H. aphrophilus* and *H. paraphrophilus* were represented by type strains, which agreed with the original descriptions on all major points. Strain J75 (=NCTC 5886) is the type strain of *H. aphrophilus* by monotypy, as it was the only strain described in the original publication of Khairat (27); it cannot therefore be replaced as atypical (5) unless evidence is adduced that it is not authentic or does not agree with the original description. There are said to be other isolations of the same strain from the same case of disease, and J120 is one of these, but it is puzzling that they appear to differ in several ways (4). There may be, therefore, some doubt on the generic authenticity of both J75 and J120, and in our hands they were very similar but not quite identical.

Two species, *H. suis* and *H. gallinarum*, raise problems that have been discussed at some length by Biberstein and White (3). *H. suis* is not a legitimate name according to Buchanan, Holt, and Lessel (8), and it should be named *H. influenzae-suis* Lewis and Shope 1931. The original isolates required both X and V factors (31, 47), but most strains from swine require only V factor (2, 63). Lewis and Shope make it clear that their strains were virtually indistinguishable from indole-negative strains of *H. influenzae*. They were nonhemolytic. It appears very likely that later isolates that required only V factor (e.g., 30) belonged to a different species, most probably a member of the *H. parainfluenzae* group represented by cluster 2. Strain J93 is recorded (36, 57) as an original strain of Lewis and Shope, but in view of the fact that it does not require X factor and possesses most of the other characteristics of cluster 2, its authenticity as a co-type strain must be considered very doubtful.

The situation with *H. gallinarum* is similar. The original organism on which this name was based was not that of de Blicke (9), although de Blicke's organism has been cited (7) as that on which the species was founded. Delaplane, Erwin, and Stuart (10) state that they believed de Blicke's organism was the same as the cultures they studied, for which they proposed the name *H. gallinarum*, but it is clear from their text that the new species was founded on an organism briefly described by Nelson (37) and on strain R.I., which they isolated themselves. They wrote: "We have not been able to study the *B. haemoglobinophilus coryza gallinarum* (DeBlicke) in our laboratory, but from certain morphological and cultural characteristics, we believe it to be identical to culture

5080 of Nelson and the R.I. strain of organisms for which we suggest the name *Hemophilus gallinarum*." In their first publication, the exact X and V requirements are difficult to infer, but the strains definitely required X factor and probably also V factor (because of the poor growth stimulation with blood that had been stored for a long period). The strains only grew well in a closed, humid container, reminiscent of the behavior of *H. paraphrophilus* (14).

In later publications (11, 12), *H. gallinarum* was found definitely to require both X and V factors and to have an obligate requirement for sodium chloride (0.5-2.5%). In view of the accumulation of CO₂ in closed containers and the sparing action of sodium chloride on the CO₂ requirement of the *H. aphrophilus* group (65), the growth requirements of *H. gallinarum* deserve deeper investigation if authentic strains can be obtained. However, other workers have isolated fowl strains that are almost indistinguishable from *H. parainfluenzae* (see reference 3 for a discussion), as are the strains we received as *H. gallinarum*. These also did not show the sodium-chloride requirement characteristic of *H. gallinarum* (7). Again, our strains cannot be regarded as authentic strains of this species.

Strain J80 (=NCTC 8479) of *H. haemolyticus* was received directly from the National Collection of Type Cultures, and evidently it is not the same as a strain bearing this number mentioned by Biberstein and Zinnemann (4) as requiring V but not X factor. In our hands J80 required both X and V factors and had more of the other characters of cluster 1 than of cluster 2, but it should be noted that the species *H. haemolyticus* Bergey et al. 1923 was founded on the beta-haemolytic "Bacillus X" of Pritchett and Stillman (40), whose exact X and V requirements were not determined. However, Stillman and Bourn (61) describe carbohydrate reactions (frequent acidity from fructose and sucrose) that are more typical of cluster 2 than of cluster 1, and J80 was exceptional among strains of cluster 1 in producing acidity from both of these. Taken in conjunction with the rather low level at which J80 joins cluster 1, this suggests that *H. haemolyticus* might be a subgroup within cluster 1.

No type strain of *Haemophilus parainfluenzae* is available, but the strains we received under this name appear to be authentic and to agree well with the original description of Rivers (43) of his two nonhaemolytic strains that required only V factor.

Fortunately many of these problems need not be faced until more is known of what

phenetic groups exist within the genus. The present investigation was seriously limited because of the relatively few strains available for study, and work on additional strains is clearly needed. There may have been a tendency in the past to establish species of *Haemophilus* on slender grounds, utilizing single characters of uncertain significance, such as haemolysis or host of origin. In some cases no strains were listed when species were set up, so that in the event of any confusion the choice of type strains is likely to be arbitrary. The present survey suggests that haemolysis is an inadequate criterion for speciation. Host of origin is never a very satisfactory criterion, and the fact that cluster 2 contains human, swine, and fowl strains that are extremely similar implies that in *Haemophilus* (as in most genera) the naming of species according to hosts is not likely to prove helpful. In our opinion names such as *H. parahaemolyticus* (39), *H. parasuis*, and *H. paragallinarum* (3) might therefore be best regarded as subjective synonyms of other names, in this instance, of *H. parainfluenzae*.

If three main centers of variation are accepted, corresponding to clusters 1, 2 and 3, these would be regarded as subgenera in the light of current taxonomic views, and this may be the best course. Nevertheless, although we still lack good criteria for deciding on the categorical rank of clusters, by comparison with the taxonomy of other groups of bacteria these three clusters would be quite appropriately treated as species. They are formed at similarity levels that are quite usual for species in other genera of bacteria, indeed rather higher than the common level of 75 to 80% (32, 54).

We know of no evidence to suggest that serological or genetic relationships between these clusters would be out of keeping with species status. As noted, fuller study may reveal subgroups representing *H. haemoglobinophilus* and some additional species. Strains of *Haemophilus* may be much more common in normal persons than is often thought (49), and new species may be found if looked for. If for the present the three clusters are treated as species, their correct names appear to be as follows: cluster 1, *Haemophilus influenzae*; cluster 2, *Haemophilus parainfluenzae*; cluster 3, *Haemophilus aphrophilus*.

We studied too few strains of the *Actinobacillus-Pasteurella* group to obtain such information about the relationships of the species of this cluster. It is well known that *Actinobacillus lignieresii* and *A. equuli* are close species, and this has been recently discussed by Ross et al. (45). The findings on the *Pasteurella* strains

studied are in fair keeping with previous numerical taxonomy on the genus (52, 60, 62), and they confirmed the distinction between *Pasteurella* and *Yersinia*. The behavior of types A and T of *P. haemolytica* Newsom and Cross in the different analyses adds to the growing evidence, reviewed by Shreeve, Ivanov, and Thompson (48), that these types may be less similar than generally thought and are possibly different species. The original strains of Newsom and Cross (38) were evidently type A according to criteria given by Shreeve et al., despite all of them being arabinose negative, so that if the types were separated into two species, type T would be the form requiring a new name.

At present the genera *Actinobacillus*, *Haemophilus*, and *Pasteurella* are included in the family *Brucellaceae* in *Bergey's Manual* (7). They are, however, phenetically very different from *Brucella* and similar genera, as we have shown elsewhere (22). They might, therefore, be segregated to form the nucleus of a new family, as they do not appear sufficiently similar to the *Enterobacteriaceae*, the other obvious possibility. Another genus that might also be associated with them is *Eikenella*, established by Jackson and Goodman (20) for the facultatively anaerobic organisms previously called *Bacteroides corrodens*, because although its GC ratio is a good deal higher, 57 to 58% (18, 20), it has a number of similarities with *Haemophilus* and *Pasteurella* as Jackson and Goodman point out. We have doubts about the authenticity of the only strain of *Noguchia* we studied (22), so the position of this genus must remain dubious at present.

ACKNOWLEDGMENTS

We thank M. J. Sackin for assistance with computing, and one of us (R. J.) acknowledges the receipt of a Scholarship from the Medical Research Council.

REPRINT REQUESTS

Address requests for reprints to: Prof. P. H. A. Sneath, Medical Research Council Microbial Systematics Unit, University of Leicester, University Road, Leicester, England.

LITERATURE CITED

1. Bergey, D. H., F. C. Harrison, R. S. Breed, B. W. Hammer, and F. M. Huntoon. 1923. *Bergey's manual of determinative bacteriology*, 1st ed. The Williams and Wilkins Co., Baltimore.
2. Biberstein, E. L., P. D. Mini, and M. G. Gills. 1963. Action of *Haemophilus* cultures on δ -aminolevulinic acid. *J. Bacteriol.* **86**:814-819.
3. Biberstein, E. L., and D. C. White. 1969. A proposal for the establishment of two new *Haemophilus* species. *J. Med. Microbiol.* **2**:75-78.
4. Biberstein, E. L., and K. Zinnemann. 1971. Report (1966-1970) of the Subcommittee on the Taxonomy of *Haemophilus* to the International Committee of Nomenclature of Bacteria. *Int. J. Syst. Bacteriol.* **21**:133-134.
5. Boyce, J. M. H., J. Frazer, and K. Zinnemann. 1969. The growth requirements of *Haemophilus paraphrophilus*. *J. Med. Microbiol.* **2**:55-62.
6. Breed, R. S., E. G. D. Murray, and A. P. Hitchins. 1948. *Bergey's manual of determinative bacteriology*, 6th ed. The Williams and Wilkins Co., Baltimore.
7. Breed, R. S., E. G. D. Murray, and N. R. Smith. 1957. *Bergey's manual of determinative bacteriology*, 7th ed. The Williams and Wilkins Co., Baltimore.
8. Buchanan, R. E., J. G. Holt, and E. F. Lessel, Jr. 1966. Index Bergeyana. An annotated alphabetic listing of names of the taxa of the bacteria. The Williams and Wilkins Co., Baltimore.
9. de Blicke, L. 1931. Een haemoglobophile bacterie als oorzaak van coryza infectiosa gallinarum. *Tijdschr. Diergeneesk.* **58**:310-314.
10. Delaplane, J. P., L. E. Erwin, and H. O. Stuart. 1934. A hemophilic bacillus as the cause of an infectious rhinitis, p. 1-12. *Agric. Exp. Sta. R.I. St. Coll. Bull. no. 244*.
11. Delaplane, J. P., L. E. Erwin, and H. O. Stuart. 1938. The effect of the X factor, of sodium chloride, and of the composition of the nutrient media upon the growth of the fowl coryza bacillus, *Hemophilus gallinarum*. *J. Agric. Res.* **56**:919-926.
12. Delaplane, J. P., and H. O. Stuart. 1941. A study of the C factor as a requirement for growth of *Hemophilus gallinarum*. *J. Agric. Res.* **63**:29-30.
13. Dunkelberg, W. E., Jr., R. Skaggs, and D. S. Kellogg, Jr. 1970. A study and new description of *Corynebacterium vaginale* (*Haemophilus vaginalis*). *Amer. J. Clin. Pathol.* **53**:370-377.
14. Frazer, J., K. Zinnemann, and J. M. H. Boyce. 1969. The effect of different environmental conditions on some characters of *Haemophilus paraphrophilus*. *J. Med. Microbiol.* **2**:563-566.
15. Gardner, H. L., and C. D. Dukes. 1955. *Haemophilus vaginalis* vaginitis. A newly defined specific infection previously classified "nonspecific" vaginitis. *Amer. J. Obstet. Gynecol.* **69**:962-976.
16. Gower, J. C. 1971. A general coefficient of similarity and some of its properties. *Biometrics* **27**:857-871.
17. Hill, L. R. 1966. An index to deoxyribonucleic acid base compositions of bacterial species. *J. Gen. Microbiol.* **44**:419-437.
18. Hill, L. R., J. J. S. Snell, and S. P. Lapage. 1970. Identification and characterisation of *Bacteroides corrodens*. *J. Med. Microbiol.* **3**:483-491.
19. Hugh, R., and E. Leifson. 1953. The taxonomic significance of fermentative versus oxidative metabolism of carbohydrates by various gram negative bacteria. *J. Bacteriol.* **66**:24-26.
20. Jackson, F. L., and Y. E. Goodman. 1972.

- Transfer of the facultatively anaerobic organism *Bacteroides corrodens* Eiken to a new genus, *Eikenella*. Int. J. Syst. Bacteriol. **22**:73-77.
21. Janssen, W. A., and M. J. Surgalla. 1968. Morphology, physiology, and serology of a *Pasteurella* species pathogenic for white perch (*Roccus americanus*). J. Bacteriol. **96**:1606-1610.
 22. Johnson, R., and P. H. A. Sneath. 1973. Taxonomy of *Bordetella* and related organisms of the Families *Achromobacteraceae*, *Brucellaceae*, and *Neisseriaceae*. Int. J. Syst. Bacteriol. **23**:381-404.
 23. Jones, D., and P. H. A. Sneath. 1970. Genetic transfer and bacterial taxonomy. Bacteriol. Rev. **34**:40-81.
 24. Judicial Commission. 1954. Opinion 13. Conservation and rejection of names of genera of bacteria proposed by Trevisan 1842-1890. Int. Bull. Bacteriol. Nomencl. Taxon **4**:151-156.
 25. Kennedy, P. C., E. L. Biberstein, J. A. Howarth, L. M. Frazier, and D. L. Dungworth. 1960. Infectious meningo-encephalitis in cattle, caused by a *Haemophilus*-like organism. Amer. J. Vet. Res. **21**:403-409.
 26. Kennedy, P. C., L. M. Frazier, G. H. Theilen, and E. L. Biberstein. 1958. A septicemic disease of lambs caused by *Hemophilus agni* (new species). Amer. J. Vet. Res. **19**:645-654.
 27. Khairat, O. 1940. Endocarditis due to a new species of *Haemophilus*. J. Pathol. Bacteriol. **50**:497-505.
 28. Kovacs, N. 1956. Identification of *Pseudomonas pyocyanea* by the oxidase reaction. Nature (London) **178**:703.
 29. Lehmann, K. B., and R. Neumann. 1896. Atlas und Grundriss der Bakteriologie und Lehrbuch der speciellen bakteriologischen Diagnostik, 2 vol. 1^e Aufl. Teil 2. Lehmann, München.
 30. Leidy, G., E. Hahn, and H. E. Alexander. 1956. On the specificity of the desoxyribonucleic acid which induces streptomycin resistance in *Hemophilus*. J. Exp. Med. **104**:305-320.
 31. Lewis, P. A., and R. E. Shope. 1931. Swine influenzae II. A hemophilic bacillus from the respiratory tract of infected swine. J. Exp. Med. **54**:361-371.
 32. Liston, J., W. Weibe, and R. R. Colwell. 1963. Quantitative approach to the study of bacterial species. J. Bacteriol. **85**:1061-1070.
 33. Mráz, O. 1969a. Vergleichende Studie der Arten *Actinobacillus lignieresii* und *Pasteurella haemolytica*. I. *Actinobacillus lignieresii* Brumpt 1910; emend. Zentralbl. Bakteriol. Parasitenk., Infektionskr. Abt. I Orig. **209**:212-232.
 34. Mráz, O. 1969b. Vergleichende Studie der Arten *Actinobacillus lignieresii* und *Pasteurella haemolytica*. II. *Pasteurella haemolytica* Newsom und Cross, 1932. Zentralbl. Bakteriol. Parasitenk., Infektionskr. Abt. I Orig. **209**:336-349.
 35. Mráz, O. 1969c. Vergleichende Studie der Arten *Actinobacillus lignieresii* und *Pasteurella haemolytica*. III. *Actinobacillus haemolyticus* (Newsom und Cross, 1932) comb. nov. Zentralbl. Bakteriol. Parasitenk., Infektionskr. Abt. I Orig. **209**:349-364.
 36. National Collection of Type Cultures 1958. Catalogue of species. Medical Research Council memorandum no. 35. Her Majesty's Stationery Office, London.
 37. Nelson, J. B. 1933. Studies on an uncomplicated coryza of the domestic fowl. I. The isolation of a bacillus which produces a nasal discharge. J. Exp. Med. **58**:289-295.
 38. Newsom, I. E., and F. Cross. 1932. Some bipolar organisms found in pneumonia in sheep. J. Amer. Vet. Ass. **80**:711-718.
 39. Pittman, M. 1953. A classification of the hemolytic bacteria of the genus *Haemophilus*: *Haemophilus haemolyticus* Bergey et al. and *Haemophilus parahaemolyticus* nov. spec. J. Bacteriol. **65**:750-751.
 40. Pritchett, I. W., and E. G. Stillman. 1919. The occurrence of *Bacillus influenzae* in throats and saliva. J. Exp. Med. **29**:259-266.
 41. Reyn, A., A. Birch-Andersen, and S. P. Lapage. 1966. An electron microscope study of thin sections of *Haemophilus vaginalis* (Gardner and Dukes) and some possibly related species. Can. J. Microbiol. **12**:1125-1136.
 42. Rivers, T. M. 1922a. Bacterial nutrition. Growth of a hemophilic bacillus on media containing only an autoclave-stable substance as an accessory factor. Johns Hopkins Hosp. Bull. **33**:149-151.
 43. Rivers, T. M. 1922b. Influenza-like bacilli. Growth of influenza-like bacilli on media containing only an autoclave-labile substance as an accessory food factor. Johns Hopkins Hosp. Bull. **33**:429-431.
 44. Rogul, M., J. J. Brendle, D. K. Haapala, and A. D. Alexander. 1970. Nucleic acid similarities among *Pseudomonas pseudomallei*, *Pseudomonas multivorans* and *Actinobacillus mallei*. J. Bacteriol. **101**:827-835.
 45. Ross, R. F., J. E. Hall, A. P. Orning, and S. E. Dale. 1972. Characterization of an *Actinobacillus* isolated from the sow vagina. Int. J. Syst. Bacteriol. **22**:39-46.
 46. Shigidi, M. A., and A. B. Hoerlein. 1970. Characterization of the *Haemophilus*-like organism of infectious thromboembolic meningo-encephalitis of cattle. Amer. J. Vet. Res. **31**:1017-1022.
 47. Shope, R. E. 1963. Porcine contagious pleuropneumonia. I. Experimental transmission, etiology, and pathology. J. Exp. Med. **119**:357-368.
 48. Shreeve, B. J., I. N. Ivanov, and D. A. Thompson. 1970. Biochemical reactions of different serotypes of *Pasteurella haemolytica*. J. Med. Microbiol. **3**:356-358.
 49. Sims, W. 1970. Oral haemophili. J. Med. Microbiol. **3**:615-625.
 50. Smith, G. R. 1959. Isolation of two types of *Pasteurella haemolytica* from sheep. Nature (London) **183**:1132-1133.
 51. Smith, G. R. 1961. The characteristics of two types of *Pasteurella haemolytica* associated with different pathological conditions in sheep. J.

- Pathol. Bacteriol. 81:431-440.
52. Smith, J. E., and E. Thal. 1965. A taxonomic study of the genus *Pasteurella* using a numerical technique. Acta Pathol. Microbiol. Scand. 64:213-223.
 53. Sneath, P. H. A. 1968. Vigour and pattern in taxonomy. J. Gen. Microbiol. 54:1-11.
 54. Sneath, P. H. A. 1972. Computer taxonomy, p. 29-98. In J. R. Norris and D. W. Ribbons (ed.), Methods in Microbiology, vol. 7A. Academic Press Inc., London.
 55. Sneath, P. H. A., and S. T. Cowan. 1958. An electro-taxonomic survey of bacteria. J. Gen. Microbiol. 19:551-565.
 56. Sneath, P. H. A., and R. Johnson. 1972. The effect on numerical taxonomic similarities of errors in microbiological tests. J. Gen. Microbiol. 72:377-392.
 57. Sneath, P. H. A., and V. B. D. Skerman. 1966. A list of type and reference strains of bacteria. Int. J. Syst. Bacteriol. 16:1-133.
 58. Snieszko, S. F., P. J. Griffin, and S. B. Friddle. 1950. A new bacterium (*Hemophilus piscium* n. sp.) from ulcer disease of trout. J. Bacteriol. 59:699-710.
 59. Sokal, R. R., and P. H. A. Sneath. 1963. Principles of numerical taxonomy. W. H. Freeman & Co., San Francisco.
 60. Stevens, M. 1969. Development and use of multi-inoculation test methods for a taxonomic study. J. Med. Lab. Technol. 26:253-263.
 61. Stillman, E. G., and J. M. Bourn. 1920. Biological study of the hemophilic bacilli. J. Exp. Med. 32:665-682.
 62. 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.
 63. White, D. C., and S. Granwick. 1963. Hemin biosynthesis in *Haemophilus*. J. Bacteriol. 85:842-850.
 64. Winslow, C. E. A., J. Broadhurst, R. E. Buchanan, C. Krumwiede, Jr., L. A. Rogers, and G. H. Smith. 1917. The families and genera of the bacteria. Preliminary report of the Committee of the Society of American Bacteriologists on characterization and classification of bacterial types. J. Bacteriol. 2:505-566.
 65. Zinnemann, K., K. B. Rogers, J. Frazer, and J. M. H. Boyce. 1968. A new V-dependent *Haemophilus* species preferring increased CO₂ tension for growth and named *Haemophilus paraphrophilus*, nov. sp. J. Pathol. Bacteriol. 96:413-419.
 66. Zinnemann, K., K. B. Rogers, J. Frazer, and S. K. Devaraj. 1971. A haemolytic V-dependent CO₂-preferring *Haemophilus* species (*Haemophilus paraphrohaemolyticus* nov. spec.). J. Med. Microbiol. 4:139-143.
 67. Zinnemann, K., and G. C. Turner. 1963. The taxonomic position of "*Haemophilus vaginalis*" [*Corynebacterium vaginale*]. J. Pathol. Bacteriol. 85:213-219.