Deoxyribonucleic Acid Characterization of a Microorganism Isolated from Infectious Thromboembolic Meningoencephalomyelitis of Cattle¹

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Deoxyribonucleic acids (DNAs) from an unnamed species of microorganism (bovine encephalitis isolate) previously placed in the family *Brucellaceae* and from representative species of this family were thermally denatured to determine mole percentages of guanine plus cytosine (mol %G+C). The results established that DNA from the bovine encephalitis isolate had a mol %G+C of 37.3 ± 0.2 , which was between values for *Haemophilus influenzae* and *Francisella tularensis*. Values for mol %G+C of the other microorganisms in this family agreed with previously reported data. A heterogeneous group of microorganisms composed of six different overlapping genera and the BEI were in the range of 33 to 46 mol %G+C. Because the mol %G+C of the genera in this group did not differ statistically, the proper taxonomic position of recognized members of the family *Brucellaceae* was questioned.

In 1956, a previously undescribed encephalitic condition of cattle termed "infectious embolic meningoencephalitis" was reported from Colorado (12). The primary encephalitic lesion was cerebral infarction. Lesions were characterized by thrombosis of blood vessels, hemorrhage, necrosis of brain tissue, and subsequent septic infection of the affected area. Streptococci and corynebacteria were isolated from several specimens and thought to be reliable cultures. However, most isolates were described as gram positive or gram negative cocci and/or bacilli, but were not identified to genus and species. A similar condition, this time involving the isolation and partial characterization of a *Haemophilus*-like organism, was reported from cattle in California in 1960 (16).

Reports of infections in a variety of syndromes, involving what was considered to be the same microorganism, have come from Illinois (8, 11), Kansas (1, 27), Iowa (6, 7), Colorado (25),

and Oklahoma (20). The microorganism has been referred to as a *Haemophilus*-like organism (20, 25), *Haemophilus somnus* (6, 7), an *Actinobacillus actinoides*-like organism (1), and an *Actinobacillus* species (11). Experimental reproduction of lesions resembling those seen in field cases of the disease has been reported by inoculation of suspensions of the microorganism into susceptible calves (1, 7, 16, 20).

During the course of a survey designed to establish the incidence, distribution, and etiology of the various encephalitides of the bovine in Kansas (W. E. Bailie, Ph.D. thesis, Kansas State Univ., Manhattan, 1969), 47 isolates of a similar microorganism were recovered from brains of 121 cattle with a similar encephalitic condition. Cultural, morphological, and biochemical examination of these isolates determined that they were essentially identical to the previously described agents (11, 16) (Tables 1 and 2). These examinations indicated that the bacterium fulfilled the requirements for inclusion in the family *Brucellaceae*, but assignment of the organism to a genus has been difficult.

Fresh isolates of the microorganism failed to grow on media not containing blood, body fluids, or certain other enrichment substances of animal origin. Examination of growth-factor requirements indicated that the microorganism

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TABLE 1. *Comparison of characteristics described for bovine encephalitis isolate, Actinobacillus species (11), and Haemophilus-like organism (16)*

Characteristic	Microorganism ^a		
	BEI	A. sp.	HLO
No. isolates examined	28	5	
Gram reaction	—	—	—
Acid-fast reaction	—	—	—
Pigment production	yel	yel	
Capsule production	—	—	—
Aerobic growth	±	±	+
Capneic growth	+	+	+
Anaerobic growth	+	+	—
Action on blood	gr	nh	nh
Catalase	—	—	—
Oxidase	+	+	+
Indole	+	+	+
Urea	—	—	—
Nitrate	+	±	+
H ₂ S paper	+	—	—
other	—	—	—
Methyl red	—	—	—
Acetylmethylcarbinol	—	—	—
Gelatin liquefaction	—	—	—
Litmus milk	—	—	red
Growth on			
Blood agar	+	+	+
Hemoglobin agar	+	+	+
MacConkey agar	—	—	—
Citrate agar	—	—	—
Enhancement of growth by			
Serum	+	+	+
Yeast product	+	+	+
Satellitic growth on			
Blood agar	—	+	—
Serum agar	—	—	+
Infusion agar	+	+	+
X factor needed	—	—	—
V factor needed	—	—	—

^a BEI, Bovine encephalitis isolate; A. sp., *Actinobacillus* species; HLO, *Haemophilus*-like organism; +, positive; —, negative; ±, questionable; yel, yellow; gr, greenish lysis; nh, nonhemolytic; red, reduction.

required an enrichment substance but that it apparently was not the classical X and/or V factor required by members of the genus *Haemophilus* (11, 16, 25). No defined coenzyme or coenzyme-like substance has been identified as the growth factor. Under the current guidelines laid down by the ICSB Subcommittee on the Taxonomy of *Haemophilus* (28), the organism does not meet the criteria for inclusion in *Haemophilus*.

It has been noted that the guanine plus cytosine content of deoxyribonucleic acid (DNA) from microorganisms has taxonomic significance (10) and that the characteristic should be included in future species descriptions (13). Hoyer and McCullough have ob-

served that accepted and proposed members of the genus *Brucella* have similar DNA base compositions and that polynucleotide homologies of species in the genus are similar (15). Because these relationships have been established for the genus *Brucella*, we anticipated that they might apply to other genera in the family *Brucellaceae*.

This study was designed to establish the guanine plus cytosine content of DNA from the bovine encephalitis isolate (BEI) and from organisms in other genera of the family *Brucellaceae* with anticipation that this charac-

TABLE 2. *Comparison of carbohydrate reactions described for bovine encephalitis isolate, Actinobacillus species (11), and Haemophilus-like organism (16)*

Carbohydrate reaction	Microorganism ^a		
	BEI	A. sp.	HLO
No. isolates examined	28	5	
O F medium			
Open	ng		
Closed	ng		
O F medium with serum			
Open	A		
Closed	A	A	
Glucose	A	A	A
Xylose	A		A
Mannitol	A		—
Lactose	—	—	—
Sucrose	—	—	— (1)
Maltose	A	A	A
Glycerol	—		
Salicin	—		
Arabinose	—		A
Adonitol	—		
Dulcitol	—		— (1)
Galactose	A		
Fructose	A		—
Mannose	A		A
Rhamnose	—		A
Trehalose	A		
Raffinose	—		—
Sorbitol	A		A
Inositol	—		—
Cellobiose	—		
Inulin	—		
Dextrin	A		
Starch	A (1)		
Melezitose	—		
Melibiose	—		
Erythritol	—		
Glycogen	A		
10% Glucose slants	ng		
10% Lactose slants	ng		
Gas from glucose	—		

^a BEI, Bovine encephalitis isolate; A. sp., *Actinobacillus* species; HLO, *Haemophilus*-like organism; ng, no growth; A, acid; —, no change; (), number of isolates deviating from majority.

teristic would provide a basis for better definition of these genera.

MATERIALS AND METHODS

Bacterial strains and cultural conditions. Typical strains of the genera selected for DNA extraction were obtained from various sources (see Table 3). Cultures of the BEI were isolated from field cases of infectious thromboembolic meningoencephalomyelitis of cattle, and representative strains were deposited in the American Type Culture Collection, Rockville, Md. under the numbers 25836, 25837, and 25838. A maximum of three transfers on an artificial medium were made. All cultures were rigorously examined for purity by staining reaction, colonial morphology, growth characteristics, and biochemical tests.

Escherichia coli, *Bacillus megaterium*, *Proteus mirabilis*, *Pasteurella multocida*, *Brucella abortus*, and *Bordetella bronchiseptica* were cultivated on Tryptose agar (Difco); *Haemophilus influenzae* was cultivated on Tryptose agar supplemented with 10% sheep blood added at 80 C, *beta* diphosphopyridine nucleotide (DPN) (2 µg/ml; Sigma Chemical Co., St. Louis, Mo.), and 1% Supplement B (Difco); *Moraxella bovis*, *Neisseria meningitidis*, *Actinobacillus lignieresii*, *Actinobacillus* sp. strain FM, and *Actinobacillus suis* were cultivated on Tryptose agar supplemented with 5% defibrinated sheep blood; *Actinobacillus equuli* was cultivated on Tryptose broth (Difco) supplemented with 5% sterile sheep serum; and *Bordetella pertussis* and the BEI were cultivated on Tryptose agar supplemented with 15% sterile defibrinated hemolyzed sheep blood. Purified agar (Difco) was added at

a 2% final concentration to solidify the media. Cultures were incubated at 37 C for 24 h. *Brucella abortus* and the BEI were incubated under increased carbon dioxide tension (5-10%); all other cultures were grown under aerobic conditions.

DNA extraction. DNA was extracted by conventional and modified conventional procedures (3, 15, 17). Bacterial cells grown on agar media were harvested with a rubber policeman while rotating the petri dish on a Spray-Fisher turntable (Fisher Scientific Co., St. Louis, Mo.). Cells of species not in the family *Brucellaceae* were then suspended in 0.15 M sodium chloride plus 0.1 M ethylenediaminetetraacetic acid (saline-EDTA) at pH 8. Cells grown in broth were harvested by centrifugation at 3440 × g, and the pellet was resuspended and washed with saline-EDTA, recentrifuged at 3440 × g, and suspended in 0.1 standard saline citrate (SSC: 0.15 M sodium chloride plus 0.015 M sodium citrate at pH 7.0 ± 0.2). Cells of organisms in genera of the family *Brucellaceae* and one harvest each of the cells of *E. coli* and *P. mirabilis* were suspended in 0.1 SSC. Self-digested Pronase (50 µg/ml; Calbiochem, Los Angeles, Calif.) and sodium lauryl sulfate (SLS) to a concentration of 1% were added to suspensions of the organisms in 0.1 SSC. Cellular digestion and lysis were accomplished by 4 h of incubation at 60 C, and the mixture was then cooled to room temperature. The concentration of SLS was brought to 2%, and 5 M sodium perchlorate (to a final concentration of 1 M) plus an equal volume of chloroform-isoamyl alcohol (24:1) were added. The mixture was shaken on a wrist-action shaker (Burrell Corp., Pittsburgh, Pa.) for 30 min, and the resulting emulsion was centrifuged at 4950 × g for 20 min. The aqueous layer was carefully decanted, placed in a

TABLE 3. Sources and DNA base compositions of strains studied

Strain	Source ^a	DNA base composition (% G+C) ^b
<i>Actinobacillus equuli</i> 9347	WRAIR	39.7 ± 0.2
<i>A. lignieresii</i> 7 strains ^c	2 sources ^c	41.1 ± 0.2
<i>A. suis</i> A8479	NCDC	40.2 ± 0.1
<i>Bacillus megaterium</i> 14581	ATCC	38.3 ± 0.2
<i>Bordetella bronchiseptica</i> A6679	NCDC	65.0 ± 0.1
<i>B. pertussis</i> 10380	ATCC	64.8 ± 0.1
<i>Brucella abortus</i> type 1 544	NADL	56.2 ± 0.2
<i>Escherichia coli</i> K-12 K12Y10F	WRAIR	50.1 ± 0.4
<i>Haemophilus influenzae</i> A6735	NCDC	38.2 ± 0.3
Bovine encephalitis isolate 7 strains ^d	KSUVDL	37.3 ± 0.2
<i>Moraxella bovis</i> 5564	NCDC	43.4 ± 0.3
<i>Neisseria meningitidis</i> NE15	WRAIR	51.6 ± 0.2
<i>Pasteurella multocida</i> A7299	NCDC	40.3 ± 0.3
<i>Proteus mirabilis</i> 9	WRAIR	38.9 ± 0.2

^a WRAIR, Walter Reed Army Institute of Research; NCDC, National Communicable Disease Center; ATCC, American Type Culture Collection; NADL, National Animal Disease Laboratory; KSUVDL, Kansas State University Veterinary Diagnostic Laboratory.

^b Results of a minimum of three thermal denaturation curves with the mean and standard deviation.

^c Strains KC591, KC213, and KC590 from NCDC, and strains NYS4222, FM, KC207, and 87A274 from WRAIR.

^d Strains 64-3411, 64-3523, 65-2624, 65-2830, 65-4060, 66-2260, and 68-4198.

narrow polypropylene test tube, and overlaid with 2 vol of 95% ethanol. The crude nucleic acid was then spooled on a glass rod by gently swirling and mixing the solutions. The collected DNA was dissolved in 0.1 SSC, 50 μ g of ribonuclease (Sigma Chemical Co., St. Louis, Mo.) per ml were added, and the mixture was incubated at 37 C for 30 min. Self-digested Pronase (15) (50 μ g/ml) was added, and the mixture was incubated for 4 h at 45 C. After the addition of SLS (to a concentration of 1%) and an equal volume of cold, distilled, water-washed phenol, the mixture was shaken at 4 C for 20 min. Chloroform-isoamyl alcohol (1 ml/25 ml) was added before shaking to help stabilize the phenol layer. The resulting emulsion was centrifuged at $12,100 \times g$ at 4 C. The aqueous layer was decanted, acetate-EDTA (3 M sodium acetate plus 0.001 M EDTA at pH 7.0) was added (1:10), and the DNA was spooled out on a glass rod with the dropwise addition of 0.54 vol of isopropanol. The collected DNA was redissolved in 0.1 SSC and held in sterile screw-capped tubes at 4 C over one to two drops of chloroform. DNA was prepared from the remaining microorganisms essentially the same way except that cells were suspended in saline-EDTA and lysed at 60 C for 10 min in the presence of 2% SLS without Pronase. *B. megaterium* was lysed with 50 μ g of crystalline lysozyme (Pentex Inc., Kankakee, Ill.) per ml for 30 min at 37 C before adding SLS and incubating at 60 C.

Determination of DNA base composition. Determinations of the DNA base ratios, expressed as the molar ratios of guanine + cytosine/adenine + thymine + guanine + cytosine $\times 100$ (mol %G+C), were performed essentially as described by Marmur and Doty (18). Thermal denaturation of DNA was accomplished at a 0.5 SSC concentration. DNA samples in 0.1 SSC were concentrated to approximately 0.5 SSC by adding 10 SSC and were equilibrated at 0.5 SSC concentration by repeated dialysis. The hyperchromic shift at 260 nm was followed with a Gilford (Gilford Instruments Laboratories, Inc., Oberlin, Ohio) recording spectrophotometer on a Beckman (Beckman Instruments, Inc., Fullerton, Calif.) model DUR monochromator with a heated sample chamber. The thermosensor was standardized with a stem-corrected National Bureau of Standards thermometer. Absorbance was corrected for thermal expansion of water. Temperature at the midpoint of absorbancy increment (T_m) was calculated as described by Marmur and Doty (18). A standard regression curve expressing the relation between mol %G+C and T_m in 0.5 SSC was derived by the least squares method (9), with average calculated T_m 's obtained from a minimum of five determinations and average literature values for mol %G+C of DNA for the following organisms: *B. abortus* (56.48), *N. meningitidis* (50.93), *E. coli* (50.37), *P. mirabilis* (39.43), *B. megaterium* (37.53), and *H. influenzae* (38.6). These organisms were selected because their DNA base compositions have been well established chemically, either by thermal denaturation or by density gradient centrifugation (2, 15, 18, 23). A minimum of three T_m determinations were made on all species. Depending on the estimated or previously determined T_m of strains examined for DNA base

composition, *E. coli* or *P. mirabilis* DNA samples in 0.5 SSC were examined in parallel as standards.

RESULTS AND DISCUSSION

Calculated values for mol %G+C of microorganisms studied are presented in Table 3. The formula for the standard regression curve derived from an average value for a minimum of five T_m 's and average values generally accepted for mol %G+C of established species was: $\%G+C = T_m - 63.54/0.47$. When the average T_m value obtained for established species was used in the derived formula, calculated values for the species were within 0.67 mol %G+C of the average values given in the literature.

To isolate DNA from members of the family *Brucellaceae*, we modified conventional methods because of problems with the BEI. Apparently, an extremely potent nuclease, inactive at 60 C and not inhibited by chelating agents or SLS, is present in the cell proteins of the BEI. After BEI cells had been suspended in saline-EDTA and lysed at 60 C for 10 min in the presence of 2% SLS and when they were being cooled to room temperature, we noted that the solution, even if cooled rapidly, lost much of its viscosity. Attempts to complete the conventional procedure were unsuccessful, and a modification was sought. Because the nuclease appeared to be inactive at 60 C, that seemed the appropriate point at which it could be inactivated. Consequently, Pronase was added to the cell suspension before adding SLS and incubating (at 60 C for an extended period) to effect digestion of the nuclease. We then could isolate what was considered to be high-quality DNA. DNA prepared from the BEI had an average A_{280}/A_{260} at 25 C of 0.534 and an average percentage of increment at A_{260} of 28.98 when thermally denatured. Values on samples of DNA isolated from *E. coli* or *P. mirabilis* by the modified method were essentially the same as values for DNA isolated by conventional methods.

Based on thermal denaturations, the value for mol %G+C for DNA from the BEI was 37.3 ± 0.2 . By placing in numerical order values (obtained in this study and from the literature) for members of this family, the BEI value fell between those for *H. influenzae* and *F. tularensis* and did not differ significantly from either (Table 4). By using the Student's *t* test, with 95% confidence intervals, 2 deg of freedom, and the largest standard deviation (0.4 mol %G+C, obtained from eight thermal denaturation profiles on three different *E. coli* DNA preparations), a difference of 3.4 mol %G+C

between two microorganisms was significant. However, because of this fact, any microorganism in this family having a mol %G+C value of 33.9-40.7 may possess relatedness to the BEI.

While this research established a position for the BEI in the sequence of values of mol %G+C for the family *Brucellaceae*, it provided and confirmed positions for other species. The value of 65.0 ± 0.1 mol %G+C obtained for DNA from *B. bronchiseptica* compared favorably with the value of 66 mol %G+C reported for that species by Hoyer and McCullough (14). *Bordetella pertussis* DNA had a value of 64.8 ± 0.1 mol %G+C, which indicated that these two organisms possibly had similar polynucleotide sequences. Their base compositions were essentially identical and significantly different from any other genera in this family. Values obtained in this study for the actinobacilli and pasteurellas also compared favorably with those reported by Bohacek and Mraz (4) for these organisms (Table 4).

The value obtained for mol %G+C for *P. multocida* was 40.3 ± 0.3 , which was approximately 3% higher than, but not significantly different from, values previously reported for that organism (2). The value was, however, significantly different from that for *P. tularensis* and supported Ritter and Gerloff (21), who hybridized *P. multocida* DNA to an extent of 0.5% relative to homologous with *P. tularensis* DNA. The two species were not considered to be closely related, and both sets of data supported the new genus *Francisella* previously proposed for *P. tularensis*.

The results of this study clarified the taxonomic standing of organisms in the genus *Bordetella*; relationships of other genera in the family remained confused. Arrangement of values for mol %G+C for *Yersinia*, *Moraxella*, *Haemophilus*, *Pasteurella*, *Actinobacillus*, BEI, and *Francisella* species in numerical order resulted in a heterogeneous group of microorganisms with a range of only 13% and much overlapping of genera (Table 4). By using a

TABLE 4. Comparison of values from the literature and obtained from thermal denaturation profiles (T_m) of guanine plus cytosine content (mol %G+C) of DNA from members of the family *Brucellaceae*

Organism	mol %G+C			
	T_m value ^a	Literature value		
		By chemistry	By T_m	By density gradient
<i>Bordetella bronchiseptica</i>	65.0 ± 0.1		66 (15)	
<i>B. pertussis</i>	64.8 ± 0.1			
<i>Brucella melitensis</i>			58 (14)	
<i>Brucella ovis</i>			58 (14)	
<i>Brucella abortus</i>	56.2 ± 0.2	57.9 (2)	57 (14), 55 (23)	
<i>Brucella neotomae</i>			57 (14)	
<i>Brucella suis</i>			57 (14)	
<i>Yersinia pestis</i>			46 (18)	
<i>Moraxella osloensis</i>				43-43.5 (5)
<i>M. bovis</i>	43.4 ± 0.3		41 (18)	
<i>M. nonliquefaciens</i>				40-42 (5)
<i>Haemophilus gallinarum</i>			42 (22)	
<i>Actinobacillus lignieresii</i>	41.4 ± 0.2		39.2-42 (4)	
<i>Pasteurella haemolytica</i>			40.8 (4)	
<i>P. multocida</i>	40.3 ± 0.3	37.6 ^b , 36.3 ^c (2)		
<i>Actinobacillus suis</i>	40.2 ± 0.1			
<i>H. aegypti</i>			40 (18)	39 (23)
<i>A. equuli</i>	39.7 ± 0.2		40.6 (18)	
<i>M. lwoffii</i>		39.4 (24)		
<i>H. suis</i>			39 (18)	
<i>H. parainfluenzae</i>			39 (18)	39 (23)
<i>H. influenzae</i>	38.2 ± 0.3	37.8 (2)	39 (18)	39 (23)
Bovine encephalitis isolate	37.3 ± 0.2			
<i>Francisella tularensis</i>		34.7 (2)	33 (18)	36 (23)

^a Results of a minimum of three thermal denaturation curves with the mean and standard deviation.

^b Identified as *Pasteurella bovisseptica*.

^c Identified as *Pasteurella aviseptica*.

significant difference of 3.4 mol %G+C, no single species was differentiated from its neighbors. It was evident either that certain microorganisms were misclassified or that DNA base composition had little taxonomic significance in classifying genera in the family *Brucellaceae*. Future studies on the similarities of the polynucleotide sequences of these microorganisms may clarify the relationships.

Organisms with closely related base compositions are believed to be phylogenetically related if they possess similar morphological, physiological, biochemical, and other characteristics (10, 19, 26). The BEI possesses many taxonomic characteristics in common with members of the genus *Haemophilus* (11, 16, 25) and, in addition, has a DNA base composition within 1% of the value obtained for *H. influenzae*. Because this microorganism is associated with specific (1, 6, 7, 12, 16, 20, 27) disease entities of cattle and has been referred to only in the vernacular, it would be highly desirable to propose a scientific name for it. The microorganism has been referred to as *Haemophilus somnus* (6, 7), as proposed by one of us (W. E. B., Ph.D. thesis), but this name has not been validly published. However, we cannot at present justify the inclusion of this microorganism in the genus *Haemophilus* as presently defined (28). Additional examination of growth factors and polynucleotide homologies may clarify the taxonomic considerations.

ACKNOWLEDGMENTS

We thank Hazel Shave and Marilyn Marshall for excellent technical assistance.

This investigation was supported by Public Health Service research grant AI-06363 from the National Institute for Allergy and Infectious Diseases. During this work, W. E. B. was the recipient of a Public Health Service special research fellowship F3-AI-33,245 from the National Institute of Allergy and Infectious Diseases.

REPRINT REQUESTS

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LITERATURE CITED

1. Bailie, W. E., H. D. Anthony, and K. D. Weide. 1966. Infectious thromboembolic meningoencephalomyelitis (sleeper syndrome) in feedlot cattle. *J. Amer. Vet. Med. Ass.* **148**:162-166.
2. Belozersky, A. N., and A. S. Spirin. 1960. Chemistry of the nucleic acids of microorganisms, p. 147-185. In E. Chargoff and J. N. Davidson (ed.), *The nucleic acids*, vol. 3. Academic Press Inc., New York.
3. Berns, K. I., and C. A. Thomas. 1965. Isolation of high molecular weight DNA from *Haemophilus influenzae*. *J. Mol. Biol.* **11**:476-490.
4. Bohacek, J., and O. Mraz. 1967. Deoxyribonucleic acid base composition of species *Pasteurella haemolytica*, *Actinobacillus lignieresii* and *Actinobacillus equuli*. *Zentralbl. Bakteriolog. Parasitenk. Infektionskr. Hyg. Abt. Orig.* **202**:468-478.
5. Bovre, K., and S. D. Henriksen. 1967. A new *Moraxella* species, *Moraxella osloensis*, and a revised description of *M. nonliquefaciens*. *Int. J. Syst. Bacteriol.* **17**:127-135.
6. Brown, L. N., R. E. Dierks, and R. C. Dillman. 1970. Problems in differential diagnosis of *Haemophilus somnus* infections ("thrombo") of feedlot cattle. *The Bovine Practitioner* **5**:36-37.
7. Brown, L. N., R. C. Dillman, and R. E. Dierks. 1970. The *Haemophilus somnus* complex. *Proc. 74th Annu. Meet. U.S.A.H.A.* 94-108.
8. Case, M. T., W. G. Raudabaugh, and J. H. Finnell. 1965. Report of embolic meningo-encephalitis in Illinois. *Ill. Vet.* **8**:31-32.
9. Freund, J. E. 1960. *Modern elementary statistics*, 2nd ed., p. 316. Prentice-Hall, Inc., Englewood Cliffs, N.J.
10. Gasser, F., and M. Mandel. 1968. Deoxyribonucleic acid base composition of the genus *Lactobacillus*. *J. Bacteriol.* **96**:580-588.
11. Gossling, J. 1966. The bacteria isolated from lesions of embolic meningo-encephalitis of cattle. *Ill. Vet.* **9**:14-18.
12. Griner, L. A., Rue Jensen, and W. W. Brown. 1956. Infectious embolic meningo-encephalitis in cattle. *J. Amer. Vet. Med. Ass.* **129**:417-421.
13. Hill, L. R. 1966. An index to deoxyribonucleic acid base compositions of bacterial species. *J. Gen. Microbiol.* **44**:419-457.
14. Hoyer, B. H., and N. B. McCullough. 1968. Homologies of deoxyribonucleic acids from *Brucella ovis*, canine abortion organisms, and other *Brucella* species. *J. Bacteriol.* **96**:1783-1790.
15. Hoyer, B. H., and N. B. McCullough. 1968. Polynucleotide homologies of *Brucella* deoxyribonucleic acids. *J. Bacteriol.* **95**:444-448.
16. Kennedy, P. C., E. L. Biberstein, J. A. Howart, 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.
17. Marmur, J. 1961. A procedure for the isolation of deoxyribonucleic acid from microorganisms. *J. Mol. Biol.* **3**:208-218.
18. Marmur, J., and P. Doty. 1962. Determination of the base composition of deoxyribonucleic acid from its thermal denaturation temperature. *J. Mol. Biol.* **5**:109-118.
19. Marmur, J., S. Falkow, and M. Mandel. 1963. New approaches to bacterial taxonomy. *Annu. Rev. Microbiol.* **17**:329-372.
20. Panciera, R. J., R. R. Dahlgren, and H. B. Rinker.

1968. Observations on septicemia of cattle caused by a *Haemophilus*-like organism. *Pathol. Vet.* **5**:212-226.
21. Ritter, D. B., and R. K. Gerloff. 1966. Deoxyribonucleic acid hybridization among some species of the genus *Pasteurella*. *J. Bacteriol.* **92**:1838-1839.
22. Rogul, M., A. Z. McGee, R. G. Wittler, and S. Falkow. 1965. Nucleic acid homologies of selected bacteria, L forms, and *Mycoplasma* species. *J. Bacteriol.* **90**:1200-1204.
23. Schildkraut, C. L., J. Marmur, and P. Doty. 1962. Determination of the base composition of deoxyribonucleic acid from its buoyant density in CsCl. *J. Mol. Biol.* **4**:430-443.
24. Sebald, M., F. Gasser, and H. Werner. 1963. Teneur en bases del' ADN et classification des vibrions. *Ann. Inst. Pasteur (Paris)* **105**:897.
25. Shigidi, M. A., and A. B. Hoerlein. 1970. Characterization of the *Haemophilus*-like organism of infectious thromboembolic meningoencephalitis of cattle. *Amer. J. Vet. Res.* **31**:1017-1022.
26. Sueoka, N. 1961. Variation and heterogeneity of base composition of deoxyribonucleic acids: a compilation of old and new data. *J. Mol. Biol.* **3**:31-40.
27. Weide, K. D., C. M. Hibbs, and H. D. Anthony. 1964. Diagnosis of bovine feedlot encephalitides. *Proc. 68th Annu. Meet. U.S. Livestock San. Ass.* 469-478.
28. Zinneman, K. 1967. Report of the subcommittee on the taxonomy of *Haemophilus*. *Int. J. Syst. Bacteriol.* **17**:165-166.