The Taxonomy of the Genera *Moraxella* and *Neisseria*

By S. D. HENRIKSEN AND K. BØVRE

Kaptein W. Wilhelmsen og Frues Bakteriologiske Institutt,
University of Oslo, Rikshospitalet, Oslo

(Accepted for publication 18 October 1967)

SUMMARY

A reclassification of the Neisseriaceae is proposed, involving: (1) Inclusion of *Moraxella* as a genus of Neisseriaceae. (2) Transfer of *Neisseria catarrhalis*, *N. ovis* and *N. caviae* to *Moraxella*, or, alternatively, creation of a new genus for these species. (3) Unification of the species *Neisseria flava*, *N. perflava* and *N. subflava* under the name *N. subflava*. (4) Unification of the species *Moraxella lacunata* and *M. liquefaciens* under the name *M. lacunata*. (5) Exclusion of the genera *Veillonella* and *Gemella* from Neisseriaceae. The relationships of the organisms *Acinetobacter anitratus* and *A. lwoffii* to the Neisseriaceae, if any, have not yet been sufficiently clarified to suggest the inclusion of these organisms in Neisseriaceae. Until this has been studied in more detail, the genus *Acinetobacter* appears to be suitable.

INTRODUCTION

The results of recent studies on the relationships of organisms belonging to the genera *Moraxella* and *Neisseria* (Bøvre, 1967; Catlin & Cunningham, 1961, 1964*a, b*) indicate that a revision of the taxonomy of these organisms is desirable. Transformation experiments and determination of DNA base composition have given particularly suggestive results. The significance of such criteria in taxonomy has been discussed elsewhere (Bøvre, 1967; Bøvre & Henriksen, 1967*a*). Here it may suffice to state that the following discussion is based upon the hypothesis that genetic homologies as demonstrated by genetic recombination between organisms have a greater significance in the taxonomy of bacteria than many phenotypic expressions which are commonly utilized in taxonomy. We also feel that the DNA base composition represents valuable supporting evidence, in the sense that wide differences between the over-all base composition of two organisms speak against close taxonomic relationship, whereas similar base compositions are compatible with, but do not prove, close relationship. At the present stage of our work we find it permissible and useful to suggest a new classification of these organisms, mainly based on the available transformation results and DNA composition data. Some of the taxonomic problems have been only tentatively solved, however. Future nucleic acid hybridization experiments may prove supporting or modifying on these points.

Relationships between Moraxella and Neisseria

Some years ago it was suggested (Henriksen, 1952) that the best choice of a family for the genus *Moraxella* might be Neisseriaceae. This suggestion was based upon similarities between the genera *Moraxella* and *Neisseria* in morphology, biochemical reactions, growth characteristics and requirements, antibiotic sensitivity and habitat. The results of transformation experiments give support to this idea. Thus transfor-

mation of relatively high efficiency, as high as or higher than between some species within each of the two genera, has been shown between *Neisseria catarrhalis*, *N. ovis* and *N. caviae* on one hand, and *Moraxella lacunata*, *M. liquefaciens*, *M. nonliquefaciens* and *M. bovis* on the other (Bøvre, 1967). Particularly high ratios between interspecific and intrastain transformant numbers (in the following such ratios are called transformation ratios for simplicity) were found in experiments with *N. ovis* and *M. bovis*. We feel that it is reasonable to assume that such high transformation ratios (up to about 0.1 %), as occurs in these cases, are indications of fairly close relationships. When this is considered in the light of the many other similarities which have been pointed out before (Henriksen, 1952), we feel that it is a sound idea to include the genus *Moraxella* in Neisseriaceae as one of its genera.

Relationships within Neisseria

Results of transformation experiments and DNA base determinations made elsewhere (Catlin, 1960, 1961; Catlin & Cunningham, 1961; Marmur, Falkow & Mandel, 1963) and on our own strain material (summarized by Bøvre, 1967) indicate that *Neisseria* species can be divided in two subgroups. One subgroup consisting of *Neisseria gonorrhoeae*, *N. meningitidis*, *N. sicca*, *N. flava*, *N. perflava*, *N. subflava*, *N. flavescens* and *N. cinerea* is characterized by guanine+cytosine (G+C) content of the DNA in the vicinity of 50 mole %. The species which have been studied show high mutual compatibility in transformation, with transformation ratios above 1 %. In the case of *N. gonorrhoeae*, this species is highly compatible with *N. meningitidis* in DNA hybridization experiments (Kingsbury & Duncan, 1967). All these organisms clearly are closely related and belong to the same genus. It might even be discussed whether they are too closely related to be separated in different species. In the case of the species *N. flava*, *N. perflava* and *N. subflava* the relationships are particularly close, with transformation ratios above 10 % in all combinations. These organisms only differ from one another in comparatively minor details, and it has always been difficult to distinguish clearly between them. It has been previously suggested that they be united in one species. We feel that the transformation data give strong support to this view, and that these species should be united. According to the data given in *Index Bergeyana* (Buchanan, Holt & Lessel, 1966) the name would have to be *N. subflava*, which appears to be the oldest legitimate name.

Neisseria gonorrhoeae, *N. meningitidis*, *N. sicca*, *N. flavescens*, and *N. cinerea* are also apparently closely related to *N. subflava* and to one another, and it might be discussed whether they should be considered as varieties of the same species rather than as separate species. However, this might cause unnecessary confusion. In particular it seems desirable to keep organisms such as *N. gonorrhoeae* and *N. meningitidis* as separate species. We suggest that also *N. sicca*, *N. flavescens* and *N. cinerea* be maintained as separate species for the time being.

The second subgroup, consisting of *Neisseria catarrhalis*, *N. ovis* and *N. caviae* have G+C contents of the DNAs in the same range as the 'classical' *Moraxella* species (*N. catarrhalis* 41–42.5 moles %) or slightly above this range (*N. ovis* and *N. caviae* 44.5–45 moles %). They show mutual compatibility in transformation, although this is low between the former and the two latter species, and also comparatively high compatibility with some *Moraxella* species. They do not attack sugars, but reduce nitrate like the majority of *Moraxella* species. In other words,

they appear to be more closely related to some *Moraxella* species than to the other subgroup of the genus *Neisseria*. For these reasons it seems sensible to separate these organisms from *Neisseria*. This might be done by transferring them to the genus *Moraxella* under the names *Moraxella catarrhalis* (with two biotypes, possibly deserving species distinction), *M. ovis* and *M. caviae*; or alternatively by creating a new genus for these species. This may be a controversial question. If genetic compatibility is to be considered more significant than phenotypic characters such as morphology, the natural choice would be to transfer these species to *Moraxella*. This also would be the simplest solution, which would avoid the creation of a new genus. On the other hand, some microbiologists may object to the inclusion of both rods and cocci in the same genus. We are in favour of the first alternative and suggest that the three species be transferred to *Moraxella*, but we feel that the second alternative might also be acceptable.

Relationships within Moraxella

Strains named *Moraxella lacunata* and *M. liquefaciens*, and most probably corresponding to these entities, were found to be highly compatible (Bøvre, 1965), with transformation ratios between 10 and 100 % in all tested combinations. The difference in conventional characters between these organisms is very slight, probably consisting in a slightly different sensitivity to certain inhibitory substances contained in some batches of peptone (Lwoff, 1947); with most of the media now in use the difference is even more difficult to demonstrate (Bøvre, 1965). Furthermore, the DNAs contain approximately the same percentages of G+C, 41.5–43 moles %. There seems to be very little reason to keep these organisms in separate species, and we propose that they be united in the species *M. lacunata*.

The species *Moraxella nonliquefaciens* and *M. bovis* appear to be very closely related to *M. lacunata* and to one another, with transformation ratios ranging from 0.1 to 1.8 %, and their DNAs show G+C values in the same range, from 40 to 43 moles %. Again it can be discussed whether these organisms should be considered as varieties of the same species or as separate species. It is true that the difference between them, both in genetic compatibility and in conventional characters, is small. But the difference is clear enough to allow easy identification. The use of varieties is slightly more cumbersome than of separate species and is rather uncommon in bacteriology. We feel that one may have to accept that the degree of relationship between the species within the same genus varies, and we can see no advantage in changing the present usage on this point. For these reasons we suggest that these three entities should remain separate species.

Two other *Moraxella* species, *Moraxella osloensis* (Bøvre & Henriksen, 1967a) and *M. phenylpyrrovica* (or *M. polymorpha*; Bøvre & Henriksen, 1967b) show slight transformation compatibility with one another. Both species are slightly compatible also with the *Neisseria* species which we have proposed to transfer to the genus *Moraxella*. *Moraxella osloensis* has revealed slight compatibility with the species *M. lacunata*, *M. nonliquefaciens* and *M. bovis*.

Moraxella osloensis and *M. phenylpyrrovica* have DNA base compositions in the same range as the former *Moraxella* species (G+C 43–43.5 moles %). They both show great likeness to *M. nonliquefaciens*, with which species they have been confused until very recently, and we feel at present that it is most sensible to keep the two species in *Moraxella*.

Moraxella kingii (Henriksen & Bøvre, 1967) differs more clearly from the other *Moraxella* species in biochemical characters, transformation and DNA base composition. This species is able to attack some carbohydrates. It appears to be completely incompatible with all other *Moraxella* species, as well as with *Neisseria catarrhalis* and *N. ovis*. It also has a slightly higher G+C content than the other oxidase positive rods (44.5 moles %). Nevertheless, for the time being it seems most reasonable to consider it as a member of the genus *Moraxella*.

Relationships to Veillonella

The genus *Veillonella* has been included in Neisseriaceae on the basis of rather superficial morphological resemblance; otherwise there is little or no similarity to *Neisseria*. The *Neisseria* and *Moraxella* species are aerobes, whereas *Veillonella* species are anaerobes. We feel that there is a fundamental difference between these two kinds of metabolism. Furthermore, there is little or no other evidence apart from the coccal shape and the, perhaps questionable, Gram-negativity of *Veillonella* (Burnett & Scherp, 1962) to indicate any relationship. We feel that there is no proof that *Veillonella* is sufficiently related to *Neisseria* to belong to the same family, and propose that it be excluded from the Neisseriaceae. It might, perhaps form a separate family, at least until its relationships have been better clarified.

Relationships to Gemella

Berger (1960a, b) showed clear differences and little resemblance between the genera *Neisseria* and *Gemella*, to which genus he removed the former *N. haemolysans*. We are of opinion that it would be better to keep *Gemella* out of the Neisseriaceae, since this would leave a more homogeneous family.

Relationships to Acinetobacter

Opinions about the relationships of the organisms variously called *Acinetobacter*, *Achromobacter*, *Mimeae*, *Moraxella*, etc., to the 'classical' *Moraxella* species are divided, some workers wishing to place these organisms in the genus *Moraxella* or at least in the same family, whereas others wish to remove these organisms to a different family, e.g. *Achromobacteriaceae*.

The results of transformation experiments do not indicate close relationship between these organisms and oxidase positive bacteria. The very slight transformation reactions obtained with DNA from *Acinetobacter* strains on *Neisseria catarrhalis*, *N. ovis* and *Moraxella osloensis* are very difficult to evaluate, and absolutely no compatibility with *Moraxella lacunata*, *M. nonliquefaciens* and *M. bovis* has been demonstrated. The relationship, therefore, if any, would appear to be distant. In view of the many other differences in conventional characters such as growth characteristics, mode of attack on sugars, oxidase reaction, antibiotic sensitivity and habitat we feel that the time is not yet ripe for the admission of these organisms to the genus *Moraxella* or to the family Neisseriaceae.

The results of the studies on these organisms which are now in progress in many laboratories may be expected to clarify the situation and should be awaited before a decision is made in this controversial matter. We conclude that these organisms should wait for admission into the Neisseriaceae until better evidence in favour of

this becomes available. In the meantime the classification of these organisms in the genus *Acinetobacter* seems to be sound, at least as a temporary measure.

*A revised classification of the Neisseriaceae, with a description
of the family and the genera*

Family Neisseriaceae Prévot, 1933

Organisms spherical in pairs with adjacent sides flattened, or rod-shaped in pairs and short chains. Organisms usually plump with diameters of $1\ \mu$ or more. Non-flagellated and often non-motile. Some species have been reported to show gliding motility. Gram-negative with some tendency to resist decolorization. Some species form yellow pigments. Aerobic. Some species prefer or require a humid atmosphere, with or without added CO_2 . Some species grow poorly or not at all in some usual media without mammalian body fluids due to sensitivity to certain inhibitory substances in peptone, but may grow in simple defined media. Optimum temperatures about $32\text{--}37^\circ$. All known species are parasitic. Give positive oxidase reaction with both dimethyl- and tetramethyl-*p*-phenylenediamine. Usually sensitive to penicillin and most other antibiotics. The type genus is *Neisseria*.

Genus I. *Neisseria* Trevisan, 1885.

Organisms coccal. Some species produce yellow pigment. Growth on standard media may be poor or fail. Biochemical activities limited. A few carbohydrates may be utilized. Indole is not produced. Nitrate not reduced. Catalase is produced. G+C content of DNA in the range about 50 moles %. Parasites of mammals. The type species is *Neisseria gonorrhoeae* Trevisan.

Species 1. *N. gonorrhoeae*.   Species 3. *N. sicca*.   Species 5. *N. flavescens*.
Species 2. *N. meningitidis*.   Species 4. *N. subflava*.   Species 6. *N. cinerea*.
(Other species which have been described, may be included.)

Genus II. *Moraxella* Lwoff, 1939

Organisms coccal or rod-shaped. Tendency to pleomorphism. No pigment. Growth on standard media may be poor or fail. Biochemical activities limited. Most species do not attack carbohydrates. Indole is not produced. Nitrate may or may not be reduced. Catalase may or may not be produced. G+C content of DNA in the range 40–45 moles %. Parasites of mammals. The type species is *Moraxella lacunata* (Eyre 1900).

Species 1. *M. lacunata* (includes the biotype *M. liquefaciens*).
Species 2. *M. nonliquefaciens* (Scarlett, 1915), Murray, 1948.
Species 3. *M. bovis* (Hauduroy *et al.* 1937), Murray, 1948.
Species 4. *M. osloensis* (Bøvre & Henriksen, 1967).
Species 5. *M. phenylpyrouvica* (Bøvre & Henriksen, 1967).
Species 6. *M. kingii* (Henriksen & Bøvre, 1967).
Species 7. *M. catarrhalis* (Frosch & Kolle, 1896), Henriksen & Bøvre, 1967.
Species 8. *M. ovis* (Lindqvist, 1960), Henriksen & Bøvre, 1967.
Species 9. *M. caviae* (Pelczar, 1953), Henriksen & Bøvre, 1967.

REFERENCES

- BERGER, U. (1960a). *Neisseria haemolysans* (Thjøtta und Bøe, 1938): Untersuchungen zur Stellung im System. *Z. Hyg. Infekt. Krankh.* **146**, 253.
- BERGER, U. (1960b). Über den Kohlehydratstoffwechsel von *Neisseria* und *Gemella*. *Zentbl. Bakt.* (I Abt. Orig.) **180**, 147.
- BØVRE, K. (1965). Studies on transformation in *Moraxella* and organisms assumed to be related to *Moraxella*. 5. Streptomycin resistance transformation between serum-liquefying, nonhaemolytic moraxellae, *Moraxella bovis* and *Moraxella nonliquefaciens*. *Acta path. microbiol. scand.* **65**, 435.
- BØVRE, K. (1967). Transformation and DNA base composition in taxonomy, with special reference to recent studies in *Moraxella* and *Neisseria*. *Acta path. microbiol. scand.* **69**, 123.
- BØVRE, K. & HENRIKSEN, S. D. (1967a). A new *Moraxella* species, *Moraxella osloensis*, and a revised description of *Moraxella nonliquefaciens*. *Int. J. system. Bact.* **17**, 127.
- BØVRE, K. & HENRIKSEN, S. D. (1967b). A revised description of *Moraxella polymorpha*, Flamm 1957, with a proposal of a new name, *Moraxella phenylpyrovuica* for this species. *Int. J. system. Bact.* **17**, 343.
- BUCHANAN, R. E., HOLT, J. G. & LESSEL, E. F. (1966). *Index Bergeyana*. Baltimore: The Williams and Wilkins Co.
- BURNETT, G. W. & SCHERP, H. W. (1962). *Oral Microbiology and Infectious Disease*, p. 299. Baltimore: The Williams and Wilkins Co.
- CATLIN, B. W. (1960). Transformation reactions within and between species of *Neisseria*. *Bact. Proc.* p. 74.
- CATLIN, B. W. (1961). Affinities among *Neisseria* as revealed by studies of DNAs and DNases. *Bact. Proc.* **90**.
- CATLIN, B. W. (1964). Reciprocal genetic transformation between *Neisseria catarrhalis* and *Moraxella nonliquefaciens*. *J. gen. Microbiol.* **37**, 369.
- CATLIN, B. W. & CUNNINGHAM, L. S. (1961). Transforming activities and base contents of deoxyribonucleate preparations from various neisseriae. *J. gen. Microbiol.* **26**, 303.
- CATLIN, B. W. & CUNNINGHAM, L. S. (1964a). Genetic transformation of *Neisseria catarrhalis* by deoxyribonucleate preparations having different average base compositions. *J. gen. Microbiol.* **37**, 341.
- CATLIN, B. W. & CUNNINGHAM, L. S. (1964b). Transforming activities and base compositions of deoxyribonucleates from strains of *Moraxella* and *Mima*. *J. gen. Microbiol.* **37**, 353.
- EYRE, J. W. (1900). A clinical and bacteriological study of diplobacillary conjunctivitis. *J. Path. Bact.* **6**, 1.
- FROSCH, P. & KOLLE, W. (1896). Die Mikrokokken. In C. Flügge: *Die Mikroorganismen*, p. 96. Leipzig: Verlag von F. C. Vogel.
- HAUDUROY, P., EHRINGER, G., URBAIN, A., GUILLOT, G. & MAGROU, J. (1937). Dictionnaire de bactéries pathogènes, p. 147. Paris: Masson et Cie.
- HENRIKSEN, S. D. (1952). *Moraxella*: classification and taxonomy. *J. gen. Microbiol.* **6**, 318.
- HENRIKSEN, S. D. & BØVRE, K. (1968). *Moraxella kingii* sp. nov., a haemolytic, saccharolytic species of the genus *Moraxella*. *J. gen. Microbiol.* **51**, 377.
- KINGSBURY, D. T. & DUNCAN, J. F. (1967). DNA homology of the genus *Neisseria*. *Bact. Proc.* p. 41.
- LINDQVIST, K. (1960). A *Neisseria* species associated with infectious keratoconjunctivitis of sheep—*Neisseria ovis* nov.spec. *J. infect. Dis.* **106**, 162.
- LWOFF, A. (1939). Revision et démembrement des Haemophilae, le genre *Moraxella* nov.gen. *Annls Inst. Pasteur, Paris* **62**, 168.
- LWOFF, A. (1947). Sur le rôle du serum dans le développement de *Moraxella lacunata* et de *Neisseria gonorrhoeae*. *Annls Inst. Pasteur, Paris* **73**, 735.
- MARMUR, J., FALKOW, S. & MANDEL, M. (1963). New approaches to bacterial taxonomy. *A. Rev. Microbiol.* **17**, 329.
- MURRAY, E. G. D. (1948). In *Bergey's Manual of Determinative Bacteriology*, 6th ed. Ed. by R. S. Breed, E. G. D. Murray and A. P. Hitchens. Baltimore: The Williams and Wilkins Co., p. 590.
- PELCZAR, M. (1953). *Neisseria caviae* nov.spec. *J. Bact.* **65**, 744.
- SCARLETT, M. (1915). Infections cornéennes à diplobacilles. Note sur deux diplobacilles non encore décrits (*Bacillus duplex non liquefaciens* et *Bacillus duplex josefi*) *Annls Oculist* **153**, 100.