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Study of the *Moraxella* Group

I. Genus *Moraxella* and the *Neisseria catarrhalis* Group[1]

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A number of strains of oxidase-positive moraxellas and of neisserias related to *Neisseria catarrhalis* were characterized with respect to a number of nutritional and physiological properties and could be assigned to several species or species groups on the basis of their phenotypic traits. This grouping was consistent with that established by Bövre on the basis of transformation frequencies for streptomycin resistance. It is proposed to reserve the generic name *Moraxella* for the oxidase-positive rod-shaped organisms, and a redescription of the genus is offered. Following the recent taxonomic proposals of Bövre and Henriksen, the specific name *Moraxella osloensis* is applied to the nutritionally unexacting strains that accumulate poly-β-hydroxybutyrate as carbon reserve. The nutritionally exacting strains are assigned to three distinct groups which can be regarded as separate species or as varieties of *M. lacunata*. The epithets applicable to these groups appear to be *lacunata*, *nonliquefaciens*, and *bovis*. The "false neisserias" could be assigned to at least three subgroups, one of which constitutes the clearly defined entity, *N. catarrhalis*, which could be distinguished from *N. caviae* and *N. ovis*.

This series of papers is concerned with the biological properties and classification of an assemblage of strictly aerobic chemoorganotrophic gram-negative bacteria to which we shall give the common name of the *Moraxella* group. These organisms are nonflaggelate cocci or rods which characteristically occur in pairs. Pigments are not produced. Within the group, there is considerable variation in minimal nutritional requirements: some members require no organic growth factors, others have growth-factor requirements of varying degrees of complexity. There is likewise considerable variation in the nature and extent of the organic compounds which can be used as principal sources of carbon and energy. The most striking common group property is a negative one: failure to grow at the expense of polysaccharides, disaccharides, polyalcohols, or (with one exception) glucose. The guanine plus cytosine (G + C) content of the deoxyribonucleic acid (DNA) is in the neighborhood of 40 to 46 moles %.

The moraxellas mimic in certain respects two other groups of gram-negative bacteria: morphologically, many of them are difficult to distinguish from the gram-negative cocci of the genus *Neisseria*; physiologically and biochemically, the more nutritionally versatile moraxellas resemble in many ways members of the genus *Pseudomonas*.

The first representative of the *Moraxella* group to be described was the Morax-Axenfeld bacillus, independently isolated from cases of human conjunctivitis by Morax (32) and Axenfeld (3). This organism was characterized as a gram-negative, nonmotile, diplo-bacillus which requires serum for growth. It was first formally named *Bacillus lacunatus* by Eyre (20). Between 1900 and 1920, a number of morphologically similar bacteria, which could, however, grow on peptone media without the addition of serum, were isolated from cases of human or bovine conjunctivitis, and described under several different specific names (24, 33, 38).

The generic position of these bacteria remained vague until in 1917 *B. lacunatus* was included in the genus *Haemophilus*, newly created by a committee of American bacteriologists as part of a sweeping nomenclatorial revision of the bacteria (46). The type species selected for the genus *Haemophilus* was *Haemophilus influenzae*. This taxonomic treatment was adopted and maintained through the first four editions of *Bergey's Manual of Determinative Bacteriology*.

[1] This report is taken, in large part, from a dissertation submitted by the senior author in partial fulfillment of the requirement for the Ph.D. Degree in Bacteriology.

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In 1939, Lwoff (29) showed that the species then assigned to the genus *Haemophilus* were heterogeneous with respect both to morphology and to nutritional requirements. He proposed the segregation of *H. lacunatus* into a new genus, *Moraxella*; and he recognized one additional species in this genus, *Moraxella duplex*, distinguishable from *M. lacunata* by the absence of a serum requirement for growth.

In 1940, Audureau (2) added a third species to the genus *Moraxella*, *M. lwoffi*. It could be differentiated from both *M. lacunata* and *M. duplex*, according to Audureau, by its ability to grow in a mineral medium with ethyl alcohol as the sole carbon source. Piéchaud, Piéchaud, and Second (34, 35), in a later study of organisms related to *M. lwoffi*, proposed one additional species, *M. glucidolytica*, for strains of nutritionally nonexacting moraxellas that were able to produce acid from glucose and other monosaccharides. The work of the French school accordingly led to the recognition of four species in the genus *Moraxella*: *M. lacunata* and *M. duplex*, both apparently nutritionally exacting to different degrees; and *M. lwoffi* and *M. glucidolytica*, which require no organic growth factors, and can be distinguished from one another by action on sugars. An additional important difference between the *lacunata-duplex* and *lwoffi-glucidolytica* subgroups was revealed by Henriksen (21) and Piéchaud (36), who discovered that the former are oxidase-positive, whereas the latter are oxidase-negative. Furthermore, the oxidase-positive subgroup is much more sensitive to penicillin than is the oxidase-negative one.

In the meantime, De Bord (19), unaware of the work of Lwoff and Audureau, had described, under the names *Herellea vaginicola* and *Mima polymorpha*, two organisms which correspond in their properties to *M. glucidolytica* and *M. lwoffi*, respectively. He also described oxidase-positive strains (equivalent to Lwoff's *M. duplex*), which he classified as *M. polymorpha* var. *oxidans*.

As we shall discuss in a later paper, the oxidase-negative moraxellas are common inhabitants of soil and water. Many of them are very versatile with respect to the range of utilizable carbon sources, as first demonstrated by Lwoff and Audureau (30) for a strain of *M. lwoffi*. Not surprisingly, therefore, these organisms have been repeatedly encountered by bacteriologists in different special contexts. Their affinity to the moraxella group has frequently not been recognized, with the consequence that they have been described under a bewildering variety of names, generic, specific, and common. Among the genera to which they have been assigned are: *Bacterium*, *Neisseria*, *Micrococcus*, *Diplococcus*, *Alcaligenes*, *Achromobacter*, *Acinetobacter*, and *Pseudomonas*.

Since 1960, genetic analysis has thrown much light on the relationships, both internal and external, of the *Moraxella* group. This work had its origin in the discovery by Alexander and Redman (1) of transformation in the genus *Neisseria*. Using streptomycin resistance as a marker, Catlin and Cunningham (15) were able to show that the species *N. meningiditis*, *N. flava*, *N. perflava*, *N. subflava*, *N. sicca*, and *N. flavescens* are readily intertransformable. The species *N. catarrhalis*, although able to undergo intraspecific transformation for streptomycin resistance, could not act either as donor or as recipient for the interspecific transformation of this character with the other *Neisseria* species listed above. Analyses of DNA composition showed, furthermore, that the DNA of *N. catarrhalis* contains approximately 41 moles % G + C whereas the other *Neisseria* have DNA with substantially higher G + C content (about 50 moles %). Accordingly, Catlin and Cunningham concluded that *N. catarrhalis* is distinct from the "true *Neisseria*."

In 1962, Bövre and Henriksen (4) demonstrated transformation of streptomycin resistance among the oxidase-positive moraxellas. Since these organisms have DNA of a base composition similar to that of *N. catarrhalis*, Bövre attempted to obtain transformation of streptomycin resistance between them, with positive results (5). This finding is fully confirmed and greatly extended by later work of Catlin and Cunningham (16–18) and by Bövre (5–13). The transformation experiments are summarized by Bövre (13). Catlin and Cunningham (17), and also Bövre (12), showed the virtual absence of transformation between the oxidase-positive and the oxidase-negative moraxellas.

Until very recently, members of the *Moraxella* group were considered to be immotile. However, in 1961, Lautrop (27) described a peculiar type of movement on solid surfaces in oxidase-negative strains, an observation extended to the oxidase-positive group by Piéchaud (37). Lautrop originally interpreted this movement as being of the gliding type found in myxobacteria; but he later reported that it can be distinguished from myxobacterial gliding movement, and proposed a new term for it: "twitching" movement (28). According to Lautrop, twitching movement also occurs in nonflagellate strains of *Pseudomonas aeruginosa* and *P. fluorescens*, but not in nonflagellate strains of *Escherichia*, *Shigella*, *Klebsiella*, and *Proteus*. The mechanism of twitching movement is at present completely obscure,

and its taxonomic significance (if any) cannot yet be assessed.

The present work represents an attempt to bring some measure of taxonomic order into the *Moraxella* group, on the basis of detailed phenotypic characterizations of many strains. Base ratio analyses of the DNA of our strains have been conducted in parallel by Mandel, and will be separately published. We have examined a total of 154 strains, including representatives of most of the previously described *Moraxella* species, both oxidase-positive and oxidase-negative, as well as strains of *Neisseria catarrhalis* and other "false neisserias." In this paper, we shall describe the properties of the oxidase-positive moraxellas, as well as of *Neisseria catarrhalis* and related forms.

MATERIALS AND METHODS

Strains assigned by us to Moraxella osloensis (Group I).

D-1 *Moraxella duplex* var. *nonliquefaciens*, strain H355 of M. Piéchaud, isolated from urine.
D-2 *Moraxella duplex* var. *nonliquefaciens*, strain H356 of M. Piéchaud, isolated from urine.
D-3 *Moraxella duplex* var. *nonliquefaciens*, strain 1905 of M. Piéchaud, isolated from a case of meningitis.
D-4 *Mima polymorpha* var. *oxidans*, strain 7349 of the E. O. King Communicable Disease Center, Atlanta, Ga., received from P. D. Mitchell.
D-5 *Mima polymorpha* var. *oxidans*, strain 7608 of the E. O. King Communicable Disease Center, Atlanta, Ga., received from P. D. Mitchell.
D-6 *Moraxella nonliquefaciens*, strain WM 36 of H. Lautrop.
D-7 *Moraxella nonliquefaciens*, strain WM 42 of H. Lautrop.
D-8 *Moraxella nonliquefaciens*, strain 19116/51 of S. D. Henriksen, received from B. W. Catlin.
D-9 *Mima polymorpha* var. *oxidans*, strain MHD 2/10 of the Milwaukee Health Department, received from B. W. Catlin; isolated from a gonorrhea-like syndrome.
D-10 *Mima polymorpha* var. *oxidans*, ATCC 10973, received from B. W. Catlin.
D-11 *Moraxella nonliquefaciens*, strain 18522/51 of S. D. Henriksen, received from B. W. Catlin.
D-12 *Moraxella nonliquefaciens*, strain 7146/51 of S. D. Henriksen, received from B. W. Catlin.
D-13 *Moraxella nonliquefaciens*, strain MHD 307 of the Milwaukee Health Department, received from B. W. Catlin; isolated from an ulcer which developed after vein stripping.
D-14 *Mima polymorpha* var. *oxidans*.

Strains assigned by us to Moraxella lacunata species group (Group II).

L-1 *Moraxella liquefaciens*, NCTC 7911, received from B. W. Catlin.
L-2 *Moraxella bovis*, NCTC 9426, received from B. W. Catlin.
L-3 *Moraxella bovis*, ATCC 10900.
L-4 *Moraxella nonliquefaciens*, strain 3828/63 of K. Bövre.
L-5 *Moraxella nonliquefaciens*, strain 4235/62 of K. Bövre.
L-6 *Moraxella nonliquefaciens*, strain 836/61 of K. Bövre.
L-7 *Moraxella nonliquefaciens*, strain 672/58 of K. Bövre.
L-8 *Moraxella nonliquefaciens*, strain 13385/62 of K. Bövre.
L-9 *Moraxella nonliquefaciens (lacunata)*, NCTC 7784.
L-10 *Moraxella bovis*, NCTC 8561.
L-11 *Moraxella bovis*, NCTC 9425.
L-12 *Moraxella lacunata* var. *typica*, strain 5493 (ATCC 17970), received from M. Piéchaud.
L-13 *Moraxella lacunata* var. *atypica*, strain 5958 (ATCC 17971), received from M. Piéchaud.
L-14 *Moraxella lacunata*, ATCC 11748.
L-15 *Moraxella lacunata*, ATCC 17951.
L-16 *Moraxella duplex* var. *liquefaciens*, ATCC 17972.
L-17 *Moraxella duplex* var. *liquefaciens*, ATCC 17956.
L-18 *Moraxella nonliquefaciens*, strain 11865/52 of S. D. Henriksen, received from B. W. Catlin.

Strains assigned by us to Neisseria catarrhalis (Group III) and to "Group IV."

N-1 *Neisseria catarrhalis*, ATCC 8176, received from A. Larson.
N-2 *Neisseria catarrhalis*, NCTC 4103, received from B. W. Catlin.
N-3 *Neisseria catarrhalis*, ATCC 8193, received from B. W. Catlin.
N-4 *Neisseria catarrhalis*, strain N9, of the University of Maryland collection, received from B. W. Catlin.
N-5 *Neisseria catarrhalis*, strain Ne13 of the New York State Department of Health; strain 34105 of B. W. Catlin, from whom it was received.
N-6 *Neisseria catarrhalis*, strain Ne23 of B. W. Catlin; from a throat culture of a healthy student.
N-7 *Neisseria catarrhalis*, strain 8313, of the University of Maryland collection, received from B. W. Catlin.
N-8 *Neisseria catarrhalis*, strain Nell, of the University of Rochester collection, received from B. W. Catlin.
N-9 *Neisseria catarrhalis*, strain 93 of U. Berger.
N-10 *Neisseria catarrhalis*, strain 748 of U. Berger.
N-11 *Neisseria catarrhalis*, strain 101 of U. Berger.
N-12 *Neisseria catarrhalis*, strain 76A of U. Berger.
N-61 *Neisseria caviae*, ATCC 14659.
N-62 *Neisseria caviae*, NCTC 10293.
N-71 *Neisseria ovis*, strain 917 of K. Lindqvist; from conjunctivitis in sheep.
N-72 *Neisseria ovis*, strain LD of K. Lindqvist; from conjunctivitis in sheep.

Stock cultures of all D strains were kept on slants of Difco Nutrient Agar, and transferred monthly. N strains and most L strains were kept on slants of Heart Infusion Agar (HIA) of the following composition: 2.5% (w/v) Difco Heart Infusion, 0.5% Difco Yeast Extract, and 2% Difco Agar (final *p*H 7.0). Strains L-12, L-13, L-14, and L-18 were kept on HIA medium with 10% (v/v) sheep serum. The N and L strains were transferred weekly, the D strains monthly. All cultures were grown at 30 C and stored at 2 C.

Methods for investigating nutritional requirements. Tests of growth factor and nitrogen requirements were made in 125-ml Erlenmeyer flasks containing 25 ml of the medium and aerated on a rotary shaker. The initial inoculum was taken from two fully grown slant cultures on complex medium. A large initial inoculum was used to allow for the possibility that only a minor fraction of the inoculated cells might be capable of growing on the test medium. A strain was considered to be capable of growth on a given medium when growth occurred through six passages using 2% (v/v) inoculum at each transfer.

All strains were tested for their ability to grow with 0.2% (w/v) sodium acetate, DL-lactate, and succinate using a mineral base which differed from that described by Stanier et al. (43) only in the use of 0.02 M phosphate buffer. Strains L-12, L-13, L-14, and L-18 were also tested in the same medium and using the same carbon sources with 30 μg/liter of oleic acid. Strains which were unable to grow on any of the above minimal media were tested for growth on three additional media of increasing complexity:

(i) *Amino acids-biotin medium (AB).* This medium was prepared by mixing aseptically the following solutions: (1) 5 g of vitamin-free casein hydrolysate, 40 mg of L-cysteine, 10 mg of L-tryptophan, 1 μg of biotin, and 0.5 g of $(NH_4)_2SO_4$ in 800 ml of water; (2) 200 ml of 0.1 M phosphate buffer, *p*H 7.0; (3) 50 mg of $MgSO_4 \cdot 7H_2O$ and 5 mg of $FeSO_4 \cdot 7H_2O$ in 2 ml of water. Solutions 1 and 2 were autoclaved separately (10 min, 15 lb/in.^{2}); solution 3 was filter sterilized. All tests were done twice using this medium with 0.1% DL-sodium lactate and 0.1% succinate.

(ii) *Amino acids-purines-pyrimidines-vitamins medium (APV).* This medium contained, in addition to the ingredients of medium AB: 20 mg/liter of adenine, guanine, cytosine, thymine, uracil, and xanthine; 2 mg/liter of choline; 1 mg/liter of 2-methyl-1,4-naphthaquinone; 0.5 mg/liter of riboflavine; 0.1 mg/liter of *p*-amino benzoic acid, folic acid, nicotinic acid, nicotinamide, pantothenic acid, pyridoxal, pyridoxine, lipoic acid, and hemin; 0.01 mg/liter of biotin and thiamine; and 1 μg/liter of vitamin B_{12}. All the vitamins were filter-sterilized. DL-Sodium lactate, 0.1%, was added to the medium.

(iii) *Amino acids-yeast extract medium (AYE).* This medium was prepared with 0.5% casein hydrolysate; 0.5% yeast extract; 0.02 M phosphate buffer, *p*H 7.0; 0.1% DL-sodium lactate; 40 mg/liter of *L*-cysteine; and 10 mg/liter of L-tryptophan. The phosphate was autoclaved separately and added aseptically to the rest of the medium.

(iv) *Heart infusion-yeast extract medium (HIB).* This medium contained 2.5% heart infusion and 0.5% yeast extract, adjusted to *p*H 7.0.

Strains L-12, L-13, L-14, and L-18 were tested for growth on HIB medium with no additions, on HIB medium with 10% sheep serum, and on HIB medium with 30 mg/liter of oleic acid. All the D strains were tested for ability to utilize nitrate as the sole nitrogen source. An acetate minimal medium was used, similar to that already described, except for the substitution of 0.2% KNO_3 and 0.02% $MgSO_4 \cdot 7H_2O$ for $(NH_4)_2SO_4$.

Methods for investigating utilization of carbon compounds. The replica plating method was used for the screening of utilizable carbon sources for all strains. For the D strains, the basal medium was the same as that used by Stanier et al. (43) and a total of 132 carbon sources were tested. For the N and L strains, which had complex nutritional requirements, complex media with a low nutrient content supplemented with the various carbon sources were used. For the N strains, the basal medium was prepared by mixing the following solutions: (1) 0.25 g of casein hydrolysate, 0.5 g of heart infusion, 0.5 g of $(NH_4)_2SO_4$ in 400 ml of water; (2) 10 g of Ionagar in 400 ml of water; (3) 200 ml of 0.1 M phosphate buffer, *p*H 7.0; (4) 50 mg of $FeSO_4 \cdot 7H_2O$ in 2 ml of water. Solutions 1, 2, and 3 were autoclaved separately (10 min, 15 lb/in.^{2}), and solution 4 was filter-sterilized. The L strains were screened on a similar medium, containing a higher concentration of heart infusion (0.1 w/v). One master plate on HIA served to print five plates, the first of which had no added carbon source, and served as a control. All plates were incubated in plastic containers to minimize evaporation. Only a limited range of potential carbon sources was tested for the N and L strains.

With the L strains, the technique described above did not always give clear-cut results. Consequently, those carbon sources which appeared to be utilized by some or all strains were rechecked with liquid media containing, in addition to the carbon source: 0.5% heart infusion, 0.1% yeast extract, and 0.02 M phosphate buffer (*p*H 7.0); for strain L-3, 1% heart infusion and 0.2% yeast extract were used. All tests were carried out in 125-ml Erlenmeyer flasks containing 25 ml of the media and incubated at 30 C on a rotary shaker. Turbidity readings were compared at intervals with those for a control flask with the basal medium. A maximal turbidity 20% above that of the control was recorded as +. A maximal turbidity 10 to 19% above that of the control was recorded as ±. The compounds tested in liquid media were: acetate, propionate, butyrate, caproate, DL-β-hydroxybutyrate, DL-lactate, pyruvate, succinate, fumarate, ethyl alcohol, *n*-proponol, *n*-butanol, L-alanine, α-ketoglutarate, L-glutamate, L-malate, L-arabinose, D-xylose, D-ribose, D-glucose, and benzoate. The utilization of carbon sources by strains L-12, L-13, L-14, and L-18 was tested only in liquid medium, using the basal medium described above, supplemented with 10% (v/v) sheep serum. The utilization of hydrocarbons by the D strains (*n*-dodecane, *n*-tetradecane, and *n*-hexadecane) was tested as described by Stanier et al. (43).

Other physiological tests. In tests for denitrification, the procedure of Stanier et al. (43) was used except that the media were prepared with 0.3% $NaNO_3$, 0.01% Ionagar, either 0.8% nutrient broth or 2.5% heart infusion, and 0.5% yeast extract. For strains N-12, N-13, N-14, and N-18, 10% sheep serum was added to the HIB medium. The cultures were observed for growth and gas production over a period of 7 days. The same medium served for tests of nitrite production, except that no agar seal was used during the incubation. The starch-iodide reagent (40) was used to

test for the presence of nitrite. Tubes which showed no nitrite after 7 days were tested for residual nirate with the diphenylamine reagent (44).

Acid production from glucose was tested both on slants and in liquid media which contained one-half of the usual concentration of either HIB or nutrient broth base, and 2 ml/liter of a 1.5% alcoholic solution of Brom Cresol Purple; the initial *p*H was 6.8. Filter-sterilized glucose was added to the autoclaved medium to give a final concentration of 2% (w/v). Observations were made after 1, 2, and 4 days of incubation.

The oxidase test was performed as described by Stanier et al. (43), and the cytochrome difference spectra were determined at liquid nitrogen as well as at room temperature.

Production of extracellular gelatinase was determined by the method described by Skerman (40). Gelatin (5%, w/v) was added to the appropriate or complex medium. The tests were done in duplicate; one set was flooded with acidic mercuric chloride after 24 hr, the other after 48 hr of incubation. Only strains giving a zone of clearing extending beyond the limits of growth were scored as positive.

Extracellular lipase production was tested by a modified procedure of Sierra (39). The appropriate complex media were supplemented with 0.01% (w/v) polyoxyethylene (24) sorbitan monooleate (Tween 80) and adjusted to *p*H 7.0. Observations were continued for 5 days.

Penicillin sensitivity was tested on plates of HIA made with 1% Ionagar, to which different amounts of penicillin G were added after cooling to 40 C. In the case of strains N-12, N-13, N-14, and N-18, 10% sheep serum was added. Cultures grown on HIB were spread as patches on the surface (four per plate). The plates were scored after 18 and 36 hr.

All D strains were examined microscopically for intracellular poly-β-hydroxybutyrate granules as described by Stanier et al. (43). In addition to DL-β-hydroxybutyrate, DL-lactate and acetate were used as carbon and energy sources. All other strains were examined after growth on complex media with DL-lactate. The nature of the reserve material was confirmed for strains D-3 and D-6 by extraction and precipitation of the polymer by the method of Williamson and Wilkinson (45). Strains N-5 and N-12 were tested for poly-β-hydroxybutyrate production as follows: exponentially growing cells were harvested by centrifugation from media containing 0.5% casein hydrolysate and 0.5% yeast extract in 0.02 M phosphate buffer, *p*H 7.0, with 0.1% of either sodium acetate or DL-lactate. The cells were suspended in 0.02 M phosphate buffer, *p*H 7.0, containing only acetate or DL-lactate. Samples of the suspension with and without the added carbon source were placed in the Warburg respirometer to follow oxygen consumption. After 3 to 4 hr, while the rate of oxygen consumption was still constant, the cells were harvested and tested for polymer by the method of Williamson and Wilkinson. A total of 400 mg (dry weight) of cells was used for each strain. Strains L-1 and L-3 were tested in an analogous fashion, except that DL-β-hydroxybutyrate was used as the substrate for growth and respiration and the growth media were more complex. For strain L-1, the medium contained 0.5% heart infusion, 0.1% yeast extract, 0.1% DL-β-hydroxybutyrate, and 0.02 M phosphate buffer (*p*H 7.0); for strain L-3, the medium contained 1% heart infusion and 0.2% yeast extract. A total of 350 mg (dry weight) of cells was used for each strain.

Morphological observations. All strains were grown in liquid media and photographed during the exponential and stationary phases of growth. The D strains were grown in nutrient broth; the L and N strains in HIB. In the case of strains L-12, L-13, L-14, and L-18, 10% sheep serum was added to the medium The dimensions of at least 10 typical cells were determined on a photomicrograph of each strain. Involution forms, where these occurred, were excluded from the measurements.

RESULTS

Oxidase reaction in the Moraxella group and its relation to the cytochrome system. The basis of the oxidase reaction in yeast was first established by Keilin (25). He demonstrated that the reagent (dimethyl-*p*-phenylenediamine) was oxidized by cytochrome *c*, while other components of the electron transport system coupled the initial oxidation with a reduction of oxygen. He concluded that the oxidase test is an indirect test for the presence of a terminal oxidase in yeast. Keilin's original conclusion has been ignored by many later workers, who have often assumed that the oxidase reaction is a direct test for a terminal oxidase of the a_3 type.

In view of the diagnostic importance of the oxidase reaction for the subdivision of the *Moraxella* group, we have determined reduced minus oxidized difference spectra for representative strains of "false neisserias," oxidase-positive moraxellas, and oxidase-negative moraxellas (Fig. 1). The false neisserias (strains N-1, N-2, N-61, N-62) and the oxidase-positive moraxellas (strains D-3, D-4, L-1, L-6) have identical difference spectra, with peaks referable to cytochromes of the *b* and *c* types. On the other hand, six representative oxidase-negative strains (28, 46, 51, 61, 90, and 94) described in a subsequent paper show no cytochrome component of the *c* type; all the peaks are referable to cytochromes of the *a* and *b* types.

The difference spectrum of oxidase-negative moraxellas is very similar to, or identical with, that of certain other oxidase-negative organisms, such as *Escherichia coli* (41) and *Pseudomonas maltophilia* (43). The difference spectrum of the oxidase-positive moraxellas resembles that of such oxidase-positive pseudomonads as *P. aeruginosa, P. fluorescens, and P. putida* (43). These observations suggest that in gram-negative bacteria, a positive oxidase test may be diag-

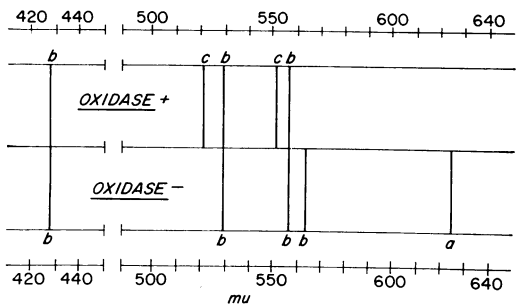

FIG. 1. *Principal peaks and shoulders of the differential reduced per oxidized cytochrome spectra of oxidase-positive and oxidase-negative strains (liquid nitrogen). The letters refer to type of cytochrome. The oxidase-positive strains used were D-1, D-3, D-4, L-1, L-6, N-1, N-61, and N-62. The oxidase-negative strains used were 28, 46, 51, 61, 90, and 94 (University of California, Berkeley, Department of Bacteriology collection).*

nostic of the presence of a cytochrome of the *c* type in the electron transport chain.

Penicillin sensitivity. The growth of all oxidase-positive moraxellas and false neisserias studied was inhibited by a concentration of 1 unit/ml of penicillin G. Of the 106 oxidase-negative strains tested, 105 were not inhibited by this concentration (Fig. 2). A similar difference in sensitivity was found for a limited number of strains of each group tested with methicillin. Several of the oxidase-negative strains were tested for possible penicillinase activity, by testing for the formation of acid from penicillin G by cell suspensions in a *p*H-stat titrator. The results were uniformly negative. These results show that penicillin sensitivity is a useful character for distinguishing between the oxidase-positive and oxidase-negative moraxellas, and, further, that the greater resistance of the latter group is not attributable to penicillinase production.

Within the oxidase-positive group, a furthur differentiation can be made on the basis of sensitivity to penicillin G at a concentration of 0.1 unit/ml. Of 14 D strains tested, 12 grow at this penicillin concentration, whereas none of the L strains and only 2 of 16 N strains can do so.

Minimal nutritional requirements for the oxidase-positive group. A basic distinction in nutritional terms can be made between the D strains and the other oxidase-positive moraxellas and false neisserias. Without exception, the D strains can grow in a basal mineral medium containing NH_4Cl as the nitrogen source, and supplemented with acetate or any other suitable single organic compound. None of the N or L strains can do so.

Eleven of the 16 N strains could grow on the

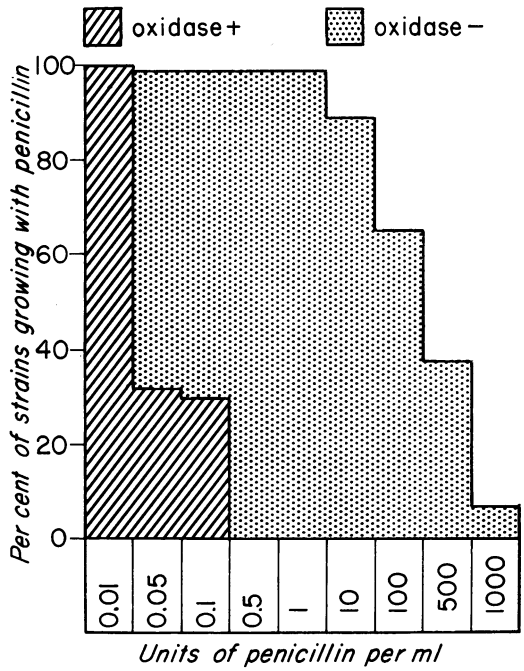

FIG. 2. *Relative resistance of oxidase-positive and oxidase-negative strains to penicillin G.*

AB medium with either lactate or succinate as principal carbon and energy source. Five N strains (N-2, N-61, N-62, N-71, and N-72) could not grow in the AB medium, nor in the APV medium, which is further supplemented with purines, pyrimidines, and additional vitamins.

The L strains were likewise heterogeneous with respect to their minimal nutritional requirements. Only one L strain (L-4) could grow on a defined medium, the very complex APV medium; removal from this medium of purines and pyrimidines, or of vitamins or amino acids prevented its growth. Strains L-1 to L-3 and L-5 to L-11 could grow well on HIB medium; strains L-12, L-13, L-14, and L-18 could not, unless either serum or oleic acid was added. Oleic acid is not the sole growth factor required by the L strains, since none of them can grow in an acetate-mineral medium supplemented with oleic acid.

The D strains were tested for their ability to use nitrate in place of ammonia as sole nitrogen source; about half the strains (D-1, D-2, D-3, D-7, D-9, D-11, D-12, and D-14) could do so.

Carbon and energy sources. Table 1 shows the range of organic compounds which can support growth of the oxidase-positive group. The full range of compounds which were tested and found

TABLE 1. *Nutritional and physiological properties of oxidase-positive moraxellas and "false neisserias"*

Group	Strains	Minimal growth factor requirement	PHB[a] accumulation	Growth with 0.1 unit of penicillin/ml	Gelatinase production	Lipase production	Nitrite production	Utilization of carbon sources: Acetate	Propionate	Butyrate	Isobutyrate	Valerate	Isovalerate	Caproate	Caprylate	Succinate	Fumarate	DL-Lactate	DL-β Hydroxybutyrate	L-Malate	Pyruvate	α-Ketoglutarate	Ethyl alcohol	n-Propanol	n-Butanol	Isobutanol	L-Alanine	L-Glutamate
I	D-1	0	−	+	−	−	−	+	+	+	+	+	+	+	−	−	−	+	+	−	+	−	+	+	+	−	−	+
	D-2	0	−	+	−	−	−	+	+	+	+	+	+	+	−	−	−	+	+	−	+	−	+	+	+	−	−	+
	D-3	0	−	+	−	−	−	+	+	+	+	+	+	+	−	−	−	+	+	−	+	−	+	+	+	−	−	+
	D-4	0	−	+	−	−	−	+	+	+	+	+	+	+	−	−	−	+	+	−	+	−	+	+	+	−	−	+
	D-5	0	−	+	−	−	−	+	+	+	+	+	+	+	−	−	−	+	+	−	+	−	+	+	+	−	−	+
	D-6	0	−	+	−	−	−	+	+	+	+	+	+	+	−	−	−	+	+	−	+	−	+	+	+	−	−	+
	D-7	0	−	+	−	−	−	+	+	+	+	+	+	+	−	−	−	+	+	−	+	−	+	+	+	−	−	+
	D-8	0	−	+	−	−	−	+	+	+	+	+	+	+	−	−	−	+	+	−	+	−	+	+	+	−	−	+
	D-9	0	−	+	−	−	−	+	+	+	+	+	+	+	−	−	−	+	+	−	+	−	+	+	+	−	−	+
	D-10	0	−	+	−	−	−	+	+	+	+	+	+	+	−	−	−	+	+	−	+	−	+	+	+	−	−	+
	D-11	0	−	+	−	−	−	+	+	+	+	+	+	+	−	−	−	+	+	−	+	−	+	+	+	−	−	+
	D-12	0	−	+	−	−	−	+	+	+	+	+	+	+	−	−	−	+	+	−	+	−	+	+	+	−	−	+
	D-13	0	−	+	−	−	−	+	+	+	+	+	+	+	−	−	−	+	+	−	+	−	+	+	+	−	−	+
	D-14	0	−	+	−	−	−	+	+	+	+	+	+	+	−	−	−	+	+	−	+	−	+	+	+	−	−	+
IIA	L-1	3	−	+	−	−	−	+	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
	L-12[a]	4	−	+	−	−	−	+	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
	L-14	4	−	+	−	−	−	+	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
	L-16	3	−	+	−	−	−	+	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
	L-17	3	−	+	−	−	−	+	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
IIB	L-2	3	−	+	−	−	−	+	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
	L-3	3	−	+	−	−	−	+	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
	L-10	3	−	+	−	−	−	+	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
	L-11	3	−	+	−	−	−	+	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
	L-15	3	−	+	−	−	−	+	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−

IIC	L-4	2	−	−	−	−	−	−	−	−	−	−	−	−	−	−	+	+	+	−	+	+	−	−	−	−	−	−	+
	L-5	3	−	−	−	−	−	+	−	−	−	−	−	−	−	−	−	+	−	+	+	−	−	−	−	−	−	−	++
	L-6	3	−	−	−	−	−	−	−	−	−	−	−	−	−	+	+	+	−	+	+	+	−	−	−	−	−	−	++
	L-7	3	−	−	−	−	−	−	−	−	−	−	−	−	−	+	+	+	−	+	+	+	−	−	−	−	−	−	++
	L-8	3	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	+	−	+	−	±	−	−	−	−	−	−	+
	L-9	3	−	−	−	−	−	−	−	−	−	−	−	−	±	±	+	−	+	+	±	−	−	−	−	−	−	−	±
	L-13	4	−	−	−	−	−	+	−	−	−	−	−	−	+	+	+	±	+	−	−	−	−	−	−	−	+	−	+
	L-18	4	−	−	−	−	−	+	−	−	−	−	−	−	−	−	+	+	−	±	−	−	−	−	−	−	+	+	+
III	N-1	1	−	−	−	+	+	+	−	−	−	−	−	−	−	+	+	+	−	+	+	±	−	−	−	−	−	−	−
	N-3	1	−	−	−	+	+	+	−	−	−	−	−	−	−	+	+	+	−	±	+	−	−	−	−	−	−	−	+
	N-4	1	−	−	−	+	+	+	−	−	−	−	−	−	−	+	+	+	−	+	+	−	−	−	−	−	−	−	++
	N-5	1	−	−	−	+	+	+	−	−	−	−	−	−	−	+	+	+	−	+	+	+	−	−	−	−	±	+	+
	N-6	1	−	−	−	±	+	+	−	−	−	−	−	−	−	+	+	+	−	+	+	+	−	−	−	−	±	+	+
	N-7	1	−	−	−	+	+	+	−	−	−	−	−	−	−	+	+	+	−	+	+	−	−	−	−	−	−	−	++
	N-8	1	−	−	−	+	+	+	−	−	−	−	−	−	−	+	+	+	−	+	+	±	−	−	−	−	+	+	++
	N-9	1	−	−	−	+	+	+	−	−	−	−	−	−	−	+	+	+	−	+	+	−	−	−	−	−	−	−	+
	N-10	1	−	−	−	+	+	+	−	−	−	−	−	−	−	+	+	+	−	+	+	+	−	−	−	−	−	+	+
	N-11	1	−	−	−	−	+	+	−	−	−	−	−	−	−	+	+	+	−	+	+	+	−	−	−	−	+	+	++
	N-12	1	−	−	−	+	+	+	−	−	−	−	−	−	±	+	+	−	±	+	+	−	−	−	−	−	−	+	++
VI	N-2	3	−	−	−	+	−	+	−	+	−	−	−	−	−	+	+	+	−	+	+	+	−	−	−	−	+	+	+
	N-61	3	−	+	−	+	+	+	−	+	−	−	−	−	−	+	+	+	−	+	+	+	−	−	−	−	+	+	++
	N-62	3	−	+	−	+	+	+	−	+	−	−	−	−	−	+	+	+	−	+	+	+	−	−	−	−	+	+	++
	N-71	3	−	−	−	+	+	+	−	+	−	−	−	+	−	+	+	+	−	±	+	+	−	−	−	−	+	+	++
	N-72	3	−	−	−	+	+	+	−	+	−	−	−	+	−	+	+	+	−	+	+	+	−	−	−	−	+	+	++

[a] Strain L-12 could not be tested for carbon source utilization because of an unidentified growth-factor requirement, which limited growth under the experimental conditions.

[b] 0 = No growth factors required; 1 = satisfied by amino acids and biotin; 2 = satisfied by amino acids, vitamins, purines, and pyrimidines; 3 = complex (e.g., heart infusion) medium required; 4 = oleic acid or serum in addition to heart infusion medium required.

[c] PHB = poly-β-hydroxybutyrate.

to be unutilizable by any strains of the D group is listed in Table 2. The italicized compounds in this table were also tested as possible substrates for the L and N strains, with negative results. Only three compounds can be used by most strains: lactate, pyruvate, and acetate. Of all groups, only lactate is universally used. The D group shows the greatest degree of nutritional versatility; most D strains can utilize C_3–C_6 fatty acids, β-hydroxybutyrate, and alcohols. However, very few D strains can utilize dicarboxylic acids (succinate, fumarate, L-malate, and α-ketoglutarate). As a group, the N strains are less versatile than the D strains, and show a nutritional pattern in many respects complementary to that of the D strains. All N strains can utilize succinate, fumarate and L-malate; none can utilize β-hydroxybutyrate and alcohols; and very few can use fatty acids of chain-length greater than C_2. As a group, the L strains are less homogeneous nutritionally than either the D or the N strains; some show a nutritional pattern resembling that of the D strains, others, a nutritional pattern resembling that of the N strains. L-5 and L-8 have the most restricted nutritional range in the entire oxidase-positive group, being able to utilize only four of the tested compounds.

TABLE 2. *Carbon sources not utilized by oxidase-positive moraxellas*[a]

Hydrocarbons, aliphatic alcohols, glycols, and polyols
n-Dodecane
n-Tetradecane
n-Hexadecane
Geraniol
Ethyleneglycol
Propyleneglycol
2,3-Butyleneglycol
Glycerol
Mannitol
Sorbitol
Adonitol
Inositol

Amino acids
Glycine
L-Alanine
β-Alanine
L-Serine
L-Valine
L-Leucine
L-Isoleucine
DL-Norleucine
L-Aspartate
L-Asparagine
L-Glutamate
L-Lysine
L-Histidine
L-Proline
L-Arginine
L-Phenylalanine
L-Tyrosine
L-Tryptophan
D-Tryptophan
L-Cystine
L-Methionine

Carbohydrates
D-Arabinose
L-Arabinose
D-Xylose
D-Ribose
Glucose
Fructose
Mannose
Galactose
Maltose
Lactose
Sucrose
Trehalose
Pectin

Amines and miscellaneous nitrogenous compounds
Ethanolamine
Butylamine
α-Amylamine
Benzylamine
Putrescine
Spermine
Histamine
Tryptamine
L-Glutamine
DL-α-Aminovalerate
L-Ornithine
L-Citrulline
Betaine
Sarcosine
Creatine
Anthranilate
Nicotinate
Pantothenate
L-Kynurenine
Kynurenate
Hippurate
Benzoylalanine
Trigonelline

Aliphatic acids
Formate
Caprate
Pelargonate
Malonate
Glutarate
Adipate
Pimelate
Suberate
Azelate
Sebacate
Eicosanedioate
Glycolate
DL-Glycerate
D-Malate
D-Tartrate
m-Tartrate
Hydroxymethylglutarate
Citrate
Aconitate
Itaconate
Mesaconate
Dimethylmalonate
Gluconate
2-Ketogluconate
Saccharate
Mucate
Poly-β-hydroxybutyrate

Nonnitrogenous aromatic and hydroaromatic compounds
o-Hydroxybenzoate
m-Hydroxybenzoate
D-Mandelate
L-Mandelate
Benzoate
p-Hydroxybenzoate
Benzoylformate
Phenylacetate
Quinate
Testosterone

[a] The D strains were tested on all of the listed substrates; the L and N strains only in the italicized compounds.

TABLE 3. *Distinguishing characteristics of the oxidase-positive moraxellas*[a]

Species group	*Moraxella osloensis*	*M. lacunata*			*Neisseria catarrhalis*		
Group number and applicable name	I *osloensis*	IIA *lacunata*	IIB *bovis*	IIC *nonliquefaciens*	III *catarrhalis*	IV *caviae*	IV *ovis*
Number of strains characterized	14	4	5	8	11	2	2
DNA composition G + C moles %[b]	43–45	42–43	41–43	41–44	40–44	46	46
Morphology exponential phase	Rods	Rods	Rods	Rods	Cocci	Cocci	Cocci
Poly-β-hydroxybutyrate accumulation	(+)	−	−	−	−	−	−
Nutritional requirements	Satisfied by mineral-acetate medium	Complex[c] (e.g., heart infusion)	Complex[c] (e.g., heart infusion)	Complex[c] (e.g., heart infusion)	Satisfied by lactate-amino acids-biotin medium	More complex than for Group III	More complex than for Group III
Gelatinase	−	+	+	−	−	−	−
Nitrite from nitrate	(−)	+	−	−	+	+	+
Resistance to 0.1 unit of penicillin G/ml	(+)	−	−	−	−	+	−
Utilization of:							
Propionate	+	−	−	−	−	−	−
Butyrate	+	+	+	−	−	+	+
Caproate	(+)	(+)	+	−	−	−	+
Ethyl alcohol	+	+ or −	+	−	−	−	−

[a] Characters designated as + or − were universally positive or negative for all strains of the group in question. Those designated as (+) or (−) were positive or negative for all but one of the strains of a given group.

[b] Determined by Manley Mandel. The values differ slightly from those published by Bövre and Henriksen (14). Considerably higher values (up to 50.4% G + C) have been published for other strains of *N. cavial* by Lamacchia and Pelczar (26).

[c] Some strains require serum or oleic acid.

Other physiological properties. All the oxidase-positive moraxellas are strict aerobes, incapable either of denitrification or of anaerobic growth in the presence of glucose. No strain can produce acid from glucose under aerobic conditions. The production of nitrite from nitrate is characteristic of the N group (one exception), occurs in some strains of the L group, and in only one strain of the D group. Gelatinase is not produced by any D or N strains; its production is variable in the L group. Lipase is produced by the majority of N strains (one exception), by all gelatinase-positive L strains, and by a few D strains (Table 1).

The D strains, with one exception (D-1), all accumulate poly-β-hydroxybutyrate as a cellular reserve material. All attempts to demonstrate its formation by L and N strains gave negative results.

Structural properties. The cells of the D and L strains are short, plump rods in the exponential phase of growth, characteristically grouped in pairs or short chains (Fig. 3, 6, 8, 10). The dimensions of exponentially growing cells range from 0.9 to 1.7 by 1.6 to 2.7 μ, with the exception of strain L-3, the cells of which are somewhat longer (3.1 to 3.3 μ). In the stationary phase, the cells retain their rod form but may become smaller (Figs. 4, 7, 9, 11); bizarre, elongated, and swollen involution forms are also frequent. The D strains may contain refractile poly-β-hydroxybutyrate inclusions (particularly prominent in nitrogen-limited cultures, Fig. 5), whereas such inclusions do not occur in L strains.

The N strains are invariably coccoid (1.0 to 1.7 μ in diameter), both in exponential and in stationary phase (Fig. 11–14). They may occur singly, in pairs, in tetrads, or in larger clumps. Continuous microscopic examination of a representative strain (N-5) showed that division regularly occurs in successive planes at approximately right-angles to one another.

DISCUSSION

Phenotypic grouping of the strains examined. The oxidase-positive strains examined in this study can be assigned to a minimum of four groups on the basis of cell shape, the nature of reserve materials, and minimal nutritional requirements (Table 1). Strains of Group I (D strains) have rod-shaped cells, accumulate poly-β-hydroxybutyrate as a reserve material, and do not require any growth factors. Strains of Group II (L strains) have rod-shaped cells, do not accumulate poly-β-hydroxybutyrate, and have complex growth factor requirements. Strains of Group III (some of the N strains, corresponding to the long-recognized species *Neisseria catarrhalis*) have coccoid cells and require amino acids and biotin as growth factors. Strains of Group IV (the remaining N strains) have coccoid cells, and their growth-factor requirements are more complex than those of Group III.

These groups can, in part, be further distinguished by additional characters. Propionate, isovalerate, valerate, *n*-propanol, *n*-butanol, and isobutanol are utilized only by strains of Group I. The two former compounds are the most useful for determinative purposes, since they are utilized by all Group I strains. Group IV differs from Group III by virtue of the ability to use butyrate as a carbon source. Resistance to penicillin G at a concentration of 0.1 unit/ml is a further character which largely distinguishes Group I from the remaining groups; with two exceptions, all Group I strains can tolerate this penicillin concentration but only two strains (both in Group IV) of the remaining three groups can do so.

Of these four major groups, Group II shows the greatest internal heterogeneity and can be divided into three subgroups, primarily on the basis of nitrate reduction, the production of gelatinase and lipase, and the ability to utilize butyrate and ethyl alcohol as carbon sources. The five strains of Subgroup IIA produce both gelatinase and lipase, and reduce nitrate to nitrite. The five strains of Subgroup IIB produce gelatinase and lipase, but do not reduce nitrate. Subgroup IIB also differs from the other groups in the ability of all strains to utilize ethyl alcohol. The eight strains of Subgroup IIC lack all of these properties. This Subgroup also has the most restricted range of carbon sources; none of the strains can utilize butyrate or caproate, which are utilized by strains of Subgroups IIA and IIB. It should be specifically noted that this subdivision of Group II shows no correlation with a possible subdivision on the basis of minimal nutritional requirements. Subgroup IIC contains the least exigent strain (L-4) of the entire group. This strain can be grown on a complex but defined medium. The Subgroup also contains two of the four most fastidious strains, which show a serum or oleic acid requirement. The other two strains (L-1 and L-12) showing this requirement fall in Subgroup IA.

Correlations with genetic studies. The phenotypic grouping which has emerged from our studies of the oxidase-positive moraxellas and false neisserias shows a remarkably good concordance with the genetic grouping established through work on transformation of the character of streptomycin resistance (13). Bövre recognized two major groups among the rod-shaped or-

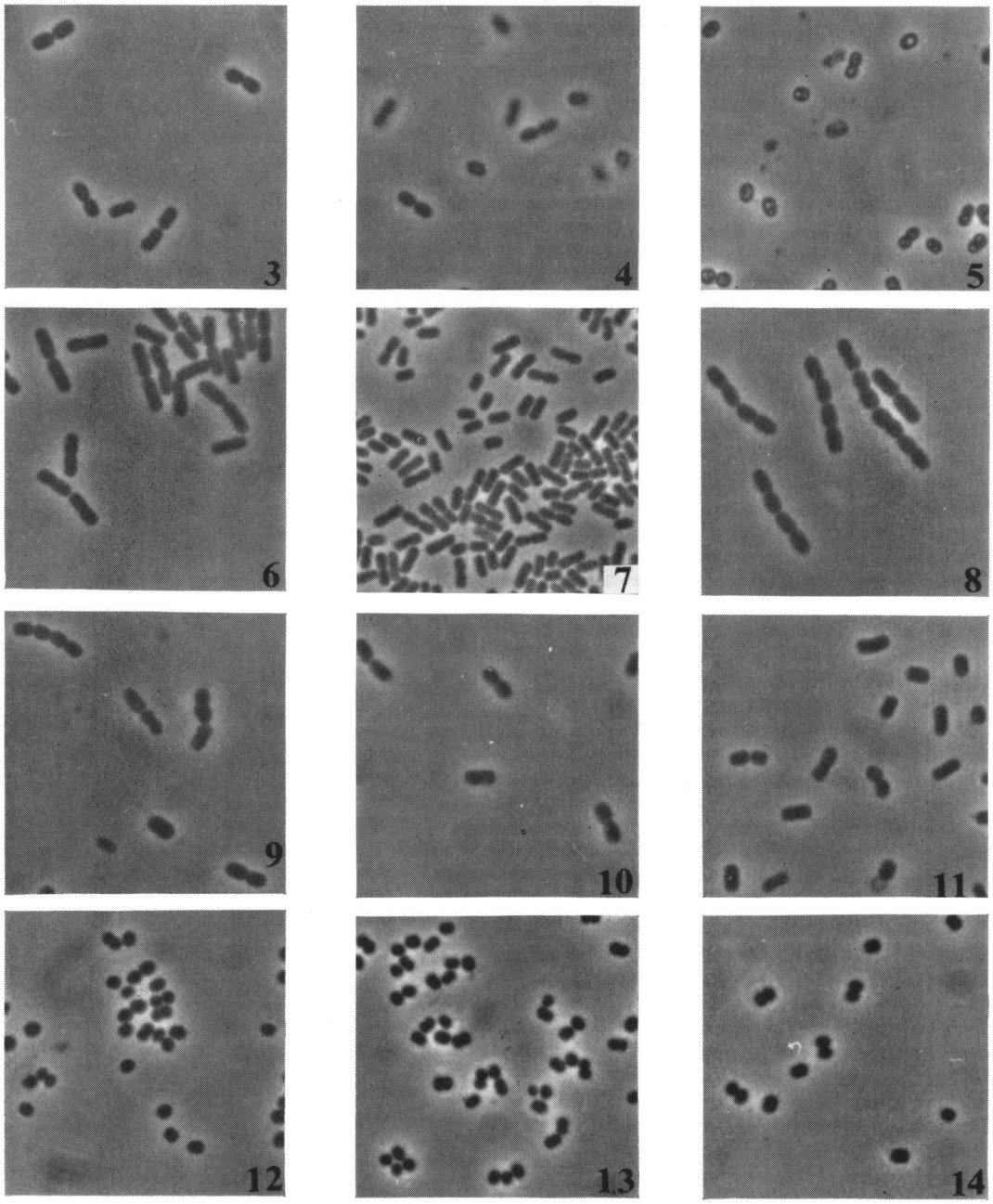

FIG. 3–14. *Phase photomicrographs of strains of moraxellas and of "false neisserias" in exponential and stationary phase of growth.* × *1,500. (3) D-8 (Group I, M. osloensis), exponential; (4) D-8, stationary; (5) D–8, grown in nitrogen-limited medium, showing poly-β-hydroxybutyrate granules; (6) L-1 (Subgroup IIA, M. nonliquefaciens), exponential; (7) L-1, stationary; (8) L-10 (Subgroup IIB, M. bovis), exponential; (9) L-10, stationary; (10) L-4 (Subgroup IIC), exponential; (11) L-4, stationary; (12) N-6 (Group III, N. catarrhalis), exponential; (13) N-6, stationary; (14) N-61 (Group IV, N. caviae), exponential. The cells were centrifuged from liquid media and resuspended in buffer.*

ganisms. Transformation could take place at varying but relatively high frequencies within each group, but only at very low frequency between the two groups. These two genetically established groups correspond to our Groups I and II. Strains of Group I which were studied by us and were shown by Bövre to be related by transformation, are: D-8, D-9, D-10, D-11, D-12, D-13 (his "19116/51" group).

Among strains that we have assigned to Group II, Bövre was able to distinguish three subgroups in terms of differences in the frequency of transformation. Specifically, he showed close genetic relationship between strains L-1 and L-14 (our Subgroup IIA); between strains L-2, L-3, L-10, and L-11 (our Subgroup IIB); and between strains L-4, L-5, L-6, L-7, and L-8 (our Subgroup IIC).

Although transformation between Groups I and II is very infrequent, some strains belonging to each of these rod-shaped groups are intertransferable (at low frequencies) with coccoid strains of Groups III and IV. Within Group III, transformation at high frequencies has been shown among strains N-3, N-4, N-5, N-6, N-7 and N-8. On the other hand, members of Group IV (N-61, N-62 and N-71) show very low transformation frequencies both to strains of Group III and to each other. In genetic terms, strain N-2 of Group IV is more closely related to Group III than it is to other members of Group IV (13). This information is summarized schematically in Fig. 15.

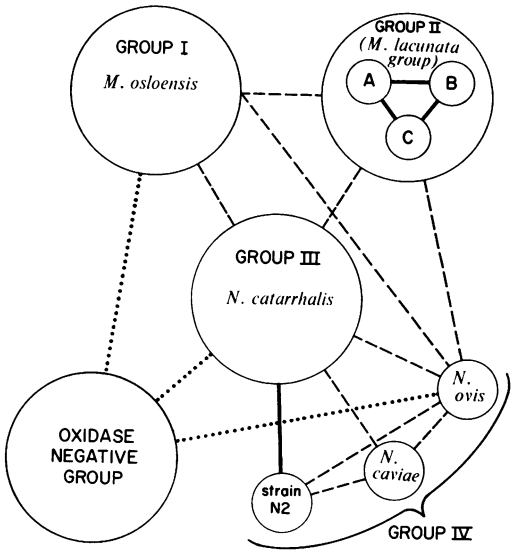

FIG. 15. *Groups and subgroups defined on the basis of phenotypic characters and their apparent genetic relationship as deduced from the studies of Catlin and Cunningham (17), Catlin (18), and of Bövre (13), and Bövre and Henriksen (14). Observed inter- to intra-strain transformation frequencies within each group or subgroup (except for oxidase-negative group) 1×10^{-1} or higher. Other inter- to intra-strain transformation frequencies as found for selected members of each group or subgroup indicated as follows: solid line, ca. 1×10^{-3} to 1×10^{-2}; broken line, ca. 1×10^{-5} to 1×10^{-3}; and dotted line, ca. 1×10^{-6} to 1×10^{-5} (semi-quantitative results of best combinations).*

Immunological work on the oxidase-positive strains studied by us are extremely limited. However, Mitchell and Burrell (31) have shown that strains D-4 and D-5 of Group I show close immunological relationships, as do strains L-1 and L-6 of Subgroups IIA and IIC, respectively; the D and the L strains are immunologically distinct.

Taxonomic considerations. On purely phenotypic grounds, a genetic separation between the oxidase-positive and oxidase-negative moraxellas can be justified. This separation is also supported by transformation experiments and by the data of John Johnson (*personal communication*) on DNA hybridization. He has found a substantial degree of genetic homology among the oxidase-negative moraxellas, but no detectable homology between these organisms and the oxidase-positive group. We therefore propose to exclude the oxidase-negative moraxellas from the genus *Moraxella*, which is defined by its oxidase-positive type species, *M. lacunata* Lwoff. We must now discuss the limits of the genus *Moraxella*. Traditionally, this genus has been restricted to rod-shaped bacteria. However, Audureau, as early as 1940 (2), emphasized that there are phenotypic resemblances between the moraxellas and *Neisseria catarrhalis*. Similarities in DNA base composition and genetic relationships between the oxidase-positive moraxellas and *N. catarrhalis* have been evident for some years. Our work has also confirmed that there are physiological resemblances among these organisms.

Since the genetic data and certain common phenotypic traits indicate that *Neisseria catarrhalis* and the related organisms of Group IV are more closely related to the rod-shaped bacteria of Groups I and II than to other species of *Neisseria*, an argument can be made for placing all of the groups studied by us in a single genus, *Moraxella*, with the necessary revision of the generic description. We believe, however, that the characterization of this genus, which would contain both rod-shaped and coccoid organisms, would cause serious practical determinative difficulties. Furthermore, we have not studied enough coccoid strains to be able to draw clear lines of distinction between the many species presently assigned to the genus *Neisseria*.

Groups I and II can both be distinguished from Groups III and IV by the permanently rod-shaped morphology of their cells, which always divide in a plane perpendicular to their long axis. These groups can also be distinguished from the oxidase-negative moraxellas by the definitely rod-shaped morphology of the cells in the stationary phase of growth, by their oxidase reaction and cytochrome spectrum, and by their relatively high sensitivity to penicillin. Furthermore, the nutritionally nonfastidious strains (Group I), which resemble the oxidase-negative organisms in their lack of growth-factor requirements, differ from the latter by their ability to accumulate poly-β-hydroxybutyrate as intracellular reserve material, by their slow growth in minimal media, and, as will be shown in a subsequent paper, by the relatively restricted spectrum of organic compounds that they can use as principal carbon sources.

We propose the following definition of the genus *Moraxella*:

Genus *Moraxella* Lwoff (emend). Nonspore-forming, nonflagellated, rod-shaped bacteria, occurring characteristically in pairs, or sometimes in short chains; gram-negative, although some strains are relatively resistant to decolorization with alcohol by comparison with such frankly gram-negative species as *Escherichia coli*. The G + C content of the DNA is in the neighborhood of 40 to 46 moles %. Oxidase positive, possessing cytochromes of the *b* and *c* types as the major components of the cytochrome system. Catalase positive. Obligately aerobic chemoorganotrophs, incapable of denitrification. Incapable of using carbohydrates, polyols, or aromatic compounds as carbon sources. Do not produce acid from glucose aerobically. Penicillin-sensitive: none can grow in the presence of 1 unit of penicillin G per milliliter.

Within the genus *Moraxella*, there are formidable nomenclatorial problems, which have been carefully analyzed by Henriksen (22, 23), and which are still not fully resolved. For example, the use of the same specific name *nonliquefaciens* for obviously different organisms by different authors has led to opposite conclusions regarding the affinity of organisms bearing this name to other oxidase-positive moraxellas.

The strains of our Group I, which show remarkable phenotypic uniformity, were received under three different taxonomic designations: *Moraxella duplex* var. *nonliquefaciens*, *Moraxella nonliquefaciens*, and *Mima polymorpha* var. *oxidans*. In a very recent publication, Bövre and Henriksen (14) have assigned a new specific name, *M. osloensis*, to this group. Although we do not particularly favor the name because it implies that the organism is specifically indigenous to Oslo, and although there may be some questions with respect to its correctness, we have applied it to our phenotypic Group I for the sake of consistency.

The strains of Group II present taxonomic as well as nomenclatorial problems. The requirement for serum (replaceable by oleic acid) which was originally used by Lwoff (29) to characterize the type species of this genus, *M. lacunata* is shown by 4 of the 18 strains which we assign to Group II. However, this characteristic is sporadically distributed in Group II, occurring in two strains assigned to Subgroup IIA, and in two strains assigned to Subgroup IIC. Since it shows no correlation with other traits that permit the division of Group II into three subgroups, it cannot be regarded as a useful taxonomic character for the definition of a species, or even of a variety. On the other hand, gelatin liquefaction and nitrate reduction, which have also been used in the past to subdivide the genus *Moraxella*, do appear to be useful characters for the internal subdivision of the strains of Group II. In conjunction with nutritional characters (carbon sources) and lipase production, they permit a division of Group II into three subgroups: A, B, and C. There appear to be two taxonomic alternatives: to regard these subgroups as separate species, or as varieties of a single species. In either event, we believe that the name *M. lacunata* (Eyre) Lwoff should be associated with the strains of Subgroup IIA. Our principal reason for considering Subgroup IIA as representing *M. lacunata* is that our strain L-12 (ATCC strain 17970) was received by us as *Moraxella lacunata* var. *typica*, strain 5493 from the collection of M. Piéchaud of the Pasteur Institute and presumably represents an authentic strain of *M. lacunata* Lwoff, isolated by Morax and Legroux in 1928 (2). Unfortunately, it could not be characterized with respect to its carbon requirements, because growth was apparently limited by an undetermined growth factor. This strain conforms to the original description of Lwoff (29) in requiring serum (or oleic acid) for growth and in liquefying gelatin. It should be noted, however, that strain L-14 (ATCC strain 11748) is a better-characterized member of this group, both phenotypically and genotypically, and also conforms to Lwoff's original description; it would, therefore, be a better neotype strain of *M. lacunata* than ATCC strain 17970. The "suggested working type" (42) of *M. lacunata*, NCTC 7784 (our strain L-9), clearly does not fall into this group, but into Subgroup IIC, on the basis of its genotypic and phenotypic characters. It appears to us that the name *M.*

liquefaciens (McNab) Murray is a synonym of *M. lacunata*, provided that the latter specific epithet remains valid.

There is little problem of assigning the name *bovis* to Subgroup IIB, since four of our five strains were so labeled and our strain L-10 is the suggested working type (42).

Subgroup IIC is the most readily distinguishable subgroup in Group II, and therefore appears to merit specific recognition if such recognition is also granted to *M. bovis* (Subgroup IIB). Bövre and Henriksen have recently redescribed this subgroup under the specific name *M. nonliquefaciens* (14). Regardless of whether the subgroup is considered as a species or as a second variety of *M. lacunata*, the name appears to be applicable to it, provided the name *osloensis* is accepted to Group I. We have, therefore, used the name *nonliquefaciens* for Subgroup IIC. Unfortunately, we did not have the proposed neotype for our phenotypic analysis.

In concluding this taxonomic analysis, we must point out that the well-established specific name *M. lacunata* is, according to Henriksen (23), a later synonym of the name *M. duplex*. However, as Henriksen himself points out, even further confusion could be created in this group by making this nomenclatorial change, and we have therefore preferred to use the better-known epithet *lacunata* for the type species of the genus.

Our limited studies of the "false neisserias" clearly characterize Group III (*N. catarrhalis*) as a phenotypically homogeneous group. The two strains received by us as *N. caviae* are sufficiently alike to represent a separate taxonomic entity, as are the two strains labeled *N. ovis*. Although the phenotypic distinctions between these subgroups are not great, there is no evidence that would counter-indicate the specific rank presently accorded them. For reasons already discussed, we are not prepared either to include these species in the genus *Moraxella*, or to create a new genus for them. It seems likely, however, that further studies on these organisms and of other neisserias will justify the assignment of the *N. catarrhalis* group to a new genus.

Table 1 summarizes the principal distinctive characteristics of the groups and subgroups studied by us.

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